

A tale of two different unions

John Maddox

Last week's state of the union messages from the leaders of the United States and the Soviet Union covered the same ground in different ways. Mr Gorbachev told the better tale but has the harder task.

DID Mr Mikhail Gorbachev stage last week's meeting of the Central Committee of the Soviet Communist Party deliberately to upstage President Ronald Reagan's State of the Union message for 1987? The constitutional calendar of the United States is both rigid and widely known, but plenary sessions of the Central Committee happen when the leadership of the Soviet Union chooses, so that the opportunity for competition for the interest of bystanders must have been apparent for some time. Naturally, it would be undignifying to suppose that great affairs of state are determined by such shallow considerations, yet Mr Gorbachev did successfully steal the limelight. No doubt we shall know if it turns out that the same happens again next year.

Meanwhile, it should be widely acknowledged that Mr Gorbachev had by far the more arresting tale to tell. Most reports of his speech in the West have centred on the proposal that party officials should in future be elected by secret ballot in which voters are given some choice, and have excited the obvious comment that much will depend on the consent of the present incumbents of these offices, as modified by instructions from above. But that issue is plainly intended only to be one practical consequence of the more general doctrine of 'democratization' that occupied much of Mr Gorbachev's speech, and that in turn was merely part of the lofty ambition that Soviet society should be transformed into one "with the most advanced economy, the broadest democracy, the most humane and lofty ethics...". None of this implies the abandonment of communism, as the frequent quotations from Lenin should have made clear, but the opposite — Mr Gorbachev's ambition that others should acknowledge that "the Bolsheviks can accomplish anything...the truth is on their side". In his view, the time has come for a radical change in the organization of Soviet society "because we just do not have the choice of another way".

Revolution

What is the chance that Mr Gorbachev can work his revolution? And what might he learn from President Reagan's address to both houses of the US Congress last week? If the president's remarks about Afghanistan, Central America and the Strategic Defense Initiative are put to one side, the two texts uncannily cover much the same ground. In the bicentenary of the US Constitution, it is natural that President Reagan should have celebrated the personal liberty that document offers, but Mr Gorbachev was also able to look forward to "socialism's most powerful creative force — free labour and free thought in a free society". On social welfare, the two men differed sharply; President Reagan, pleading for a balanced budget without extra taxes, had no arithmetical choice but to advocate a reduction of welfare spending; Mr Gorbachev, by contrast, begins with the recognition that it cannot be before 2000 that every Soviet family has an apartment of its own, which of necessity must be built with state capital. But on the relationship between research, education and economic growth, there is an identity of interests: both the president and the general secretary promised more attention to the education of the young and a more effective framework for the application of science to industrial needs.

Of the two men, Mr Gorbachev has by far the harder task. So

much is clear from his open admission last week of the failures of past years and by his no doubt accurate explanation that the inefficiency of the Soviet system is rooted in its failure, by the denial of personal responsibility, fully to engage people's interest and thus enthusiasm in the work on which they are engaged. But, by the same test, he has by far the greater opportunity. Simply by untying people's hands, it might be possible to engineer a couple of decades of economic growth comparable in speed with those of Japan in the 1960s and 1970s. True, even then the Soviet Union would still have some catching up to do, but in transformations of the kind he is attempting, people are often content to know that they are travelling in the right direction. The immediate obstacle is the sheer inertia of the Soviet bureaucracy, perhaps reinforced by the ill-will of those who now enjoy its favours. The question of whether, further down the road Mr Gorbachev would tread, will emerge the obstacle that has given pause to Mr Deng Xiaoping's modernization of China, that of accommodating diverse personal inclinations within the framework of a monolithic state, is in one sense academic; time will tell empirically whether Bolshevism is best. But if the momentum of change should be seen to flag, the tendency to dissidence will be anticipated. Mr Gorbachev has a narrow tightrope in front of him.

Reform

How well will Soviet science serve his purpose? As with the Soviet economy as a whole, recent history is largely that of opportunity postponed, sometimes flatly rejected. Mr Gorbachev last week promised both educational reform and both Party and government support for the "interesting ideas and proposals" of the new president of the Academy of Sciences, G.I. Marshuk, without saying what these are. But it is not difficult to guess. Too many of the Academy's institutes are in the hands of people who have long since ceased to be creative scientists, or who qualify for Mr Gorbachev's structures of those who abuse their office to keep others in thrall or merely in ignorance. That is one field for fertile reform. Another is the sharp gulf, recently a little less wide, between university teachers and professional researchers. Yet another is the difficulty of changing from one field of interest to another, or even simply of moving to another institute. More serious still, as a brake on the effectiveness of Soviet science, is the wide and largely unspoken separation between civil and military research. Simply in organization, there is a huge stable-cleaning to be done.

The danger, in the Soviet Union as elsewhere, is that even Mr Gorbachev's supporters will be tempted to behave as if it is simply an administrative matter to harness research to the cause of economic growth. By now the whole world knows that the most durable form of technology transfer is the slowest — the physical transfer of well-trained people with an appetite for making industry function efficiently. The more immediate stratagems, such as the "new science and technology centers" that President Reagan was promising last week, can be either stop-gaps or devices for influencing the personal inclinations of talented people, but they do not sufficiently cater for the need



identified by many others than Mr Gorbachev. So how, in the brave new Soviet Union, will people be guided to those activities best suited to the interests of the greater good when their inclinations might take them elsewhere? Mr Gorbachev's answer last week was that democracy at the workplace (and in the academy institutes) may do the trick. Many of those who have read what he had to say with enthusiasm will no doubt give him the benefit of such doubts as they may have. Everything will depend on whether he and his managers of research can deliver the promise before it is forgotten, or overwhelmed by cynicism. □

Star Wars for arms *John Maadley*

This week's resumption of arms control negotiations should be interesting, even productive.

CURIOUS things are happening about arms control. Both the United States and the Soviet Union appear to be eager to negotiate with each other, as if they were back together at Reykjavik in the early northern winter, while being as far apart as they were then found to be on what they are negotiating about. It is as if the medium has taken control of the message, the process of the product. The reason, of course, is that neither of the superpowers dares face the risk of giving lasting offence to the constituencies for a more rational way of conducting international affairs, while neither feels able to compromise what it considers to be crucial interests. So the Soviet Union is busily dismantling the phased-array radar at Krasnoyarsk, in Siberia, for fear of being held in violation of the 1972 treaty on Anti-Ballistic Missiles (ABM) while the United States appears to have encouraged its negotiators to talk about the conditions under which ABM would be applicable to the Strategic Defense Initiative (SDI) against the wishes of the Pentagon. It is not unusual that the two governments should be talking to each other without quite knowing why, but there may be profit for the rest of us in the circumstances.

SDI remains the stumbling-block, as it is bound to be. Nearly four years have passed since the concept of a shield against other people's attacks was put forward in the United States; it is natural that the passage of time should wear away at everybody's peace of mind. The effect is most noticeable in the United States, where the Department of Defense and its supporters have been wondering aloud, as if in a private soliloquy, whether to deploy some elements of this shield against hostile missiles long before the due date, sometime towards the end of the century. The objective is to ensure that there is something tangible in place before the end of the present administration, so that its successors will feel impelled along the track of their predecessor by the weight of money spent. But it may be a late even for that.

The plain truth is that the United States can serve no useful purpose by talking about the early deployment of SDI unless it plans to withdraw the undertaking given to the British prime minister three years ago that there would be no deployment without consultation with the other side, presumed to be the Soviet Union. The undertaking has been an important part of the cement that has held Western Europe together with itself in the past four years, and surprisingly tolerant of United States policy on other matters at the same time. The trouble is that the talk of installing something under the umbrella of SDI sooner rather than later, even if it might have been installed ten years ago, is bound to seem a violation of that undertaking. Whatever the technical *lacunae* of the interceptor phases of SDI may be, that fact remains that the United States cannot seriously pretend to deploy elements of SDI without losing friends in Western Europe.

Confusion arises because the Soviet Union knows this as well as the United States, yet appears willing to open the bargaining in this round at Geneva without acknowledging as much. One explanation of this week's events is that the Soviet Union is

willing to talk out the two years ahead in the hope of having a more constructive conversation with the next president; the error in that calculation is that the next president of whatever stripe will need time to become acclimatized to his environment. Another is that the Soviet Union has decided that, with no prospect of another agreement on arms control, it will serve somebody's purpose that there should be a clear understanding of what ABM really means. Yet another is that the talk is merely talk for the sake of talking, to avoid the contumely of being seen to give up.

But this is wide of everybody's mark. What people want, just now, is a simple agreement that both sides will reduce the potency of their strategic forces by some verifiable amount, by a number of warheads appropriately weighted or by a proportion not dissimilar to a half. There is no lack of frameworks of agreement, nor any danger for either side that one agreement would become a bar to others. With such modest objectives, many agreements nearly reached in recent years would suffice. Salt II, never ratified, was technically violated last year (by the United States), when an extra bomber was commissioned, yet is still within one per cent of balance. Why not the same at half the level? Is that too much to ask for as a temporary solution for a continuing problem? □

Changing British sports *John Maadley*

British universities seem to have learned one transatlantic lesson — professional athleticism.

It is a long time since the British shared the belief that others have of them, that their favourite sports are fox-hunting, horse-racing, cricket and rowing, in that order. (To judge by times allocated in broadcasting schedules, the most popular game is now snooker, played with a number of differently coloured balls on a billiards table.) It is also, as others well know, some time since the British had a university system that could rub shoulders on equal terms with university systems elsewhere still prolific of scholarship of the highest quality. The British themselves may not yet accept the second of these propositions, which may help to explain why the University of Oxford has for several weeks been preoccupied by the contentious question of which eight students should represent it in next month's boat race against the University of Cambridge, traditionally held on the neutral water filling a four-mile stretch of the tidal Thames in west London. Why else should a university whose survival is threatened by lack of funds indulge the luxury of brooding on such a trivial matter?

The issue has many ramifications, not least of which appears to be Anglo-American tension of a familiar kind. The circumstances are that, after a long winning streak, Oxford lost last year's boat race and promptly set about ensuring that the same would not happen again by recruiting from the United States a number of stalwart rowing men as graduate students, some reading for diplomas in social science (one of Oxford's softer options) who had already shown their promise as oarsmen, often by winning prizes in competitions for the US universities at which they had been undergraduates. Trouble seems to have arisen when one of these stalwarts, a veteran of last year's crew, was excluded from this year's boat. For the best part of two weeks, the Oxford crew has declined to practise, putting at risk even its loaded chance of success. The quarrel was patched up only at the weekend, with the recalcitrants returning to the fold. Now, whatever the outcome of next month's race, it is clear that Oxford has in some sense lost. The race rules are that rowers must be students, but it has not previously been found necessary to define what being a student means. Now Cambridge will be within its rights to demand some kind of definition. People elsewhere may reflect that, in the decline of great universities, external pressure not merely drives away the good but brings out the worst in those who are left. □

Soviets seek Japan's help over Chernobyl accident

- Soviet team visits atomic war site
- Hiroshima researchers devise programme

London

THE Soviet Union is planning a major survey of the medical consequences of the Chernobyl disaster based on a health monitoring programme drawn up by the Radiation Effects Research Foundation at Hiroshima, Japan. A Soviet team of five, which has just spent 10 days in Japan at the request of the Soviet side, was presented with the monitoring programme at the end of its stay.

The object of the monitoring programme will be to monitor, and attempt the prognosis and treatment of, the long-term effects on those exposed to radiation around and near Chernobyl. As part of the programme, an attempt will be made to determine the doses of radiation to which people were exposed.

The last question is important. Except for the inhabitants of the town of Pripyat, who were evacuated the day after the accident on 27 April last year, other people in the 30-km zone around Chernobyl were not moved until several days after the accident, although radioactivity continued to escape from the damaged reactor until 8 May.

Soviet medical scientists at the post-accident review conference at Vienna in August, 1986, spoke in terms of an eventual few thousand extra deaths from cancer among the 135,000 persons from the 30-km zone, but estimates of this order seemed to be little better than 'best guesses'. Soviet participants at Vienna indicated that, then, they were by no means certain of the levels of contamination of individuals either within the 30-km zone or beyond.

At the same meeting, the Soviet delegation indicated that the best memorial to those who had died and those who were yet to die as a result of the Chernobyl accident would be to wring all possible information from the disaster. The question of whether or not there is a minimum exposure below which radiation produces no harmful effects was singled out for attention.

The logistics of such a follow-up programme are formidable. Not only will it entail the lifelong monitoring of the inhabitants of the 30-km zone, but it will also require the selection of an appropriate control population.

The latter will undoubtedly find regular check-ups increasingly tedious as the years pass, but there may be some difficulty in matching the control subjects for

comparable lifestyle while, at the same time, going sufficiently far from Chernobyl to ensure that contamination of the control subjects is no more than background contamination.

Nevertheless, the Soviet team declared in Vienna that a survey would be carried out once plans had been drawn up; a training programme for medical personnel was to have been launched immediately. The visit to Hiroshima, and the handing of the Japanese monitoring programme, suggest that the follow-up programme is well on the way to becoming a reality. The Soviet physicians told their Japanese hosts that the results of the study would be "announced internationally" for the benefit of world medicine.

Japan is undoubtedly best fitted by history to provide the details of a monitoring programme, although there is one respect in which Japanese experience and that of the Soviet monitoring teams are not entirely comparable. The survivors of Hiroshima and Nagasaki had not been exposed to contamination by heavy radionuclides, whereas much of the long-term radiation exposure consequent on Chernobyl is likely to be caused by inhaled or ingested radioactivity.

A particular consequence of the accident now being brought to light is the possibility that 'micro-hotspots' consisting of small particles of radioactivity may have been more widely distributed after Chernobyl than first seemed possible. Although the amount of the fuel which 'relocated' in areas remote from the reactor site was only a minute fraction of the whole, 'micro-hotspots' were recorded as far afield as Sweden in the first reports of radioactivity after the accident.

Although the early Soviet medical work after Chernobyl concentrated on radioiodine and caesium, recent Soviet reports obliquely indicate that hotspot contamination is causing increasing concern. No details of heavy nuclide contamination within the Soviet Union is available so far (apart from anecdotal evidence from the clothes and baggage of returning tourists, see *Nature* 323, 399; 1986), but a Polish nuclear safety expert, who preferred not to be identified, said in October that whole-body monitoring suggests that as many as one in ten inhabitants of Warsaw has ruthenium hotspots in his or her lungs, while in Bialystok, close to the frontier of the Byelorussian SSR, the proportion may be as high as one in three. **Vera Rich**

British nuclear industry in trouble again

London

BRITAIN'S nuclear lobby is under fire in the wake of the disclosure that the Sellafield reprocessing plant in Cumbria is to be closed down for two weeks because of a leak and reports that the United Kingdom's advanced gas-cooled reactors (AGR) have a design fault.

These incidents are likely to revive the opposition to the building of the £1,300 million pressurized water reactor (PWR) at Sizewell on the Suffolk coast, despite tacit approval for its construction by a report published last week. The (see *Nature* 325, 377 and 378; 1987) study by Sir Frank Layfield has been criticized by, among others, two major environmental lobbyists, the Friends of the Earth and the Council for the Protection of Rural England, which say the study avoids issues and is irrelevant after Chernobyl.

The council was scathing. It claimed that "since the inquiry closed, events have moved on radically. Not only have the all-important cost of other fuels — coal and oil — fallen through the floor, but also the Central Electricity Generating Board's (CEGB, operators of the plant) own estimate of what Sizewell B will cost to build has soared, by more than 11 per cent (£127 million), before even a brick has been laid."

But the leaks at Sellafield and the alleged AGR design fault will do more damage than any protest from ecologists to the nuclear case. Sellafield management was warned by the Nuclear Installations Inspectorate (NII) the safety watchdog of the nuclear industry and the body responsible for implementing the conditions of the nuclear operating licences, that the plant would be closed within a year if safety recommendations were not carried out.

The latest leak is the third in the past three weeks and will mean the complex will have to close for up to two weeks. The AGR design faults, believed to be a weakness in the control rods, will also apply to the two reactors being built at Heysham, near Blackpool and Torness, Scotland, at a cost of £1,000 million each.

These disclosures will undermine the credibility of the Layfield study. While the PWR design is significantly different from that of AGR, the Layfield survey emphasizes that not all the safety aspects of the PWR design have been tested, nor could they be at this stage. The long-term safety of the nuclear design depends on assurances given by CEGB and NII, an admission that shocked many opponents of CEGB and NII. **Bill Johnstone**

US high-energy physics plans hot up with supercollider go-ahead

Washington

THE jewel in the high-energy physics crown, the Superconducting Super Collider (SSC), took a step closer to reality last week. Energy Secretary John Herrington announced last Friday that President Reagan is giving full support to building SSC, and that the administration will ask for the funds to start the \$4,400 million project in 1988.

Final presidential approval for the project came after a meeting of the Domestic Policy Council last Thursday. Although Herrington had announced last year that he supported the project (see *Nature* 324, 502; 1986), the White House has been conspicuously silent. There was, for example, no mention of the project in the 1988 budget request last month nor in the president's State of the Union address last week.

Herrington said a decision on how a site for SSC will be selected will be announced on 10 February. Details of how the project will be financed will also then be spelled out. Herrington speculates that the 1988 budget request for SSC will be approximately \$60 million.

Presidential approval by no means makes construction of SSC a certainty. Congress must be convinced that SSC's scientific and political rewards outweigh the financial obligations.

SSC is seen as necessary for the next generation of high-energy physics. The present generation of accelerators has produced proton-antiproton collisions at energies up to 1.8 million million electron volts (1.8 TeV), but SSC will make possible proton-proton collisions at energies up to 40 TeV, when fundamental properties of matter now hidden should be revealed by peaks in the interaction cross-section of the particles and in the products of their collisions.

To achieve such high energies, SSC will have a main ring 82.94 km in circumference lined with approximately 10,000 superconducting magnets. SSC will consist of two concentric rings, each capable of accelerating protons up to 20 TeV. There will be four points along the accelerator circumference where the two beams can intersect, allowing collisions. The design for SSC was coordinated by the Central Design Group, at Lawrence Berkeley Laboratory in California. The Department of Energy will be the principal federal agency in the project. The Energy Department now provides 90 per cent of the support for high-energy physics research in the United States.

Although the decision to build SSC is unilateral, Herrington said the United States plans to "seek maximum cost-

sharing funding from other countries", as well as contributions from private industries and local governments. But whatever the level of international enthusiasm, Herrington stresses that SSC is "an American project, with American leadership, and we're going forward with it".

Choosing a site for SSC is bound to be a political free-for-all. More than half the states in the Union have announced an interest in the project, some spending millions of dollars to make an attractive bid. The state that wins the prize will reap an economic windfall from the construction



Fermilab director Leon Lederman faces the inevitable cash shortage.

of a project the size of SSC, not to mention enhanced prestige.

But even as the Reagan administration turns its sights towards the future of high-energy physics, two new machines are getting ready to start full-scale operations. At Fermi National Accelerator Laboratory (Fermilab) outside Chicago, the Tevatron has begun producing proton-antiproton collisions after a shutdown for repairs.

In the fall of 1985, the Collider Detector at Fermilab (CDF) recorded the first 1.8-TeV collisions produced by the Tevatron. Now a 3-m central tracking chamber just commissioned will allow precise tracking of particles produced in collisions. The Tevatron produced low-intensity collisions last December, and now both CDF and Tevatron are being debugged. So far, says to Fermilab's Hans Jensen, beam luminosity has been "quite modest", but he expects that will improve by the time full power operations begin in March.

The other new machine expected to begin operations this year is the Stanford Linear Collider (SLC) in Palo Alto, California. According to project administrator Don Getz, both positron and electron beams have reached design energies of 50 thousand million electron volts (50 GeV), and he expects both beams will be into the

Johns Hopkins atop spending table

Washington

THE table below shows the top ten US universities ranked by federal research funds received in fiscal year 1985, based on data in a recent report from the National Science Foundation. Johns Hopkins owes its ranking to the \$299 million a year that went to its Applied Physics Laboratory, a mostly independent defence research facility counted in the university total only since 1978. Similar independent research facilities at the Massachusetts Institute of Technology, Stanford University and Cornell University are not included in the totals.

US university research and development during 1985

Institution	Federal funds (\$ million)
Johns Hopkins University	429.18
Massachusetts Institute of Technology	187.65
Stanford University	189.96
University of Washington	146.18
University of California, Los Angeles	128.21
Columbia University (main division)	127.33
University of Wisconsin, Madison	124.60
Cornell University	119.97
Harvard University	109.41
Yale University	109.23

Most federal funds for research in 1985 came from 15 agencies. Topping that list is the Department of Health and Human Services (\$3,098.6 million), followed by the Department of Defense (\$1,040.8 million), then the National Science Foundation (\$1,011.1 million) and the Department of Energy (\$373.1 million). Total federal spending on research and development in universities and colleges in 1985 was \$6,379.2 million. Joseph Palca

final focus by the end of February. The primary detector will be installed in April, and Getz hopes to start seeing Z bosons in May. Getz says SLC will begin operating at a luminosity well below design capability, but even at 1,000 times below design specifications he expects 15-20 Zs a day.

Funds will be a problem for both facilities. Getz admits that the money available will not allow SLC to be operated as much as he would like. Leon Lederman, Fermilab's director, also faces difficult financial decisions in operating the Tevatron.

Although enthusiasm for SSC abounds throughout the high-energy physics community, physicists are anxious that its financing should be truly independent from continuing projects. Alvin Tollestrup, co-leader of CDF, says SSC is the "obvious next step" for the future. But, says Tollestrup, siphoning funds from experiments already in place to pay for SSC would be "a disaster". Joseph Palca

President Reagan offers strong prescription for a healthy nation

Washington

ROCKED by political controversy and medical problems, President Ronald Reagan needed to put on a strong performance in his annual State of the Union message delivered last week to Congress. Most agree he did, but many felt he was strong on style and short on substance.

Reagan spent little time discussing the Iran arms scandal, even though it is still in the headlines nearly every day. Instead, he dwelt on familiar topics: support for the Contras in Nicaragua; support for the Strategic Defense Initiative; and criticism of Congress for the "sorry spectacle" of the budget process. Reagan promised to provide to Congress new plans for restoring US competitiveness, including new science and technology centres and "strong new funding" for basic research.

Documents released by the White House to accompany Reagan's speech provided a few more details about how the president plans to achieve renewed competitiveness. Protection of intellectual property, investment in education and research, legal and regulatory reforms, and reducing the budget deficit are all part of the prescription. The president proposes a "Technology Share" programme between federal agencies and consortia of private industry and universities. There will also be a push to the new Federal Technology

Transfer act designed to encourage economic development of ideas hatched at national laboratories.

The president renewed his commitment to the US space station, the hypersonic aerospace plane and a global geoscience programme. He also supports a plant science programme to be funded by the National Science Foundation (NSF) and the Departments of Energy and Agriculture. New technology centres, already established in engineering and biology by NSF, are also part of the formula.

Science education will receive one of the largest increases if the president gets his way. NSF's budget for education increases by 50 per cent in 1988 to \$273 million. In addition to graduate and post-graduate support, NSF will also be turning its attention to improving undergraduate science instruction. The 1988 NSF budget calls for \$68 million for undergraduate programmes, up \$38 million from the 1987 appropriation. NSF also announced last week a new initiative in science education for 5- to 18-year-old students. The new programme makes NSF funds available to academic publishers for developing coherent science curricula for all the pre-university years. Publishers will match the NSF funds — \$6.6 million in the first round of awards — and put 5 per cent of profits into teacher education.

Joseph Palca

Spanish take French lessons

Barcelona

INSPIRED by the success of the French student riots, Spanish students have taken to the streets in a similar push for reform in education. Their protests, however, have become increasingly violent.

It was left to the Education and Science Minister José Maria Maravall, to conduct the negotiations with the student representatives in an attempt to allay their fears and reach a compromise on the proposed educational reforms.

There are several differences between the Spanish unrest and that in France. Those protesting in Spain were mainly 14



José Maria Maravall — pondering unrest.

to 18 year olds and they opposed two main features of current educational policy introduced over the past three years — the increase in the tuition fees for universities and the introduction of an entrance selection system. Previously, after secondary school, any student could freely enter any faculty in any university.

But the consequences of the 'baby-boom' in the 1960s created an explosion in the number of university students. Although the number of universities has doubled in the past 20 years, very little attempt has been made to increase the quality of teaching. One solution, popular with university staff but not with students, was to introduce selection. There is now a two-part system in which secondary school results are taken into account with the results of an entrance examination.

Another element in the reform was to allow the universities more autonomy by increasing the tuition fees. After years of relatively modest fees, each student now pays the equivalent each year of half the monthly salary of a middle-class worker.

The new proposals of the Minister of Education and Science to quell the unrest are an increase in the funding for secondary schools; an increase in the number of fellowship and gratuities offered to students from low-income families; freezing of tuition fees; and reform of the university selection procedure.

Pedro Puigdoménech

World Health Organisation plans better AIDS management

London

THE World Health Organisation (WHO) has set this year's budget for combating AIDS (acquired immune deficiency syndrome) at US\$43.7 million and has launched an international study to survey trends in AIDS legislation. By the end of the year, the budget will allow about 32 people, twice as many as now, to work full-time on monitoring the disease.

WHO wants to establish itself as the "worldwide clearing house for reliable information on AIDS". To that end, one of its newest and most important research projects is the global survey examining the trends in AIDS legislation. The results of the study would be expected to be presented to an international forum of public health and legal experts whose brief would be to provide guidelines on the alternative approaches to the notification and reporting of AIDS and human immunodeficiency virus (HIV) infection, the protection of blood supplies and donation of organs.

Most legislation so far is concentrated in North America, Australia and Europe,

although the highest incidence of the disease is in Africa. There is growing pressure on WHO from its member states to consider AIDS as a global crisis and to ensure that management to combat the disease is centralized, although each country would be self-reliant in fighting the disease.

France has already taken the initiative in legislation. President François Mitterrand has signed the law that will sweep away the taboos that have prevented the advertising of condoms, considered vital in combating the spread of AIDS. Part of the £20 million health campaign under way in Britain, both in posters and the 20-million-plus home leaflets being distributed nationally, emphasizes the use of condoms but there are severe restrictions on the public advertising of condoms. The same taboos apply in the United States.

The West German government is fighting off the opponents of its public health campaign, part of a DM20 million programme, where newspaper advertising is being used to call for more extensive use of condoms.

Bill Johnstone

West German commission reports on genetic engineering

Hamburg

WIDE-ranging recommendations on the applications of genetic engineering are contained in a report of a West German parliamentary commission. Although generally approving the use and exploitation of genetic manipulation, the commission calls for a ban on experiments on fertilized human eggs that have the potential to develop into "complete human individuals" and for a five-year moratorium on the environmental release of genetically transformed microorganisms.

The 400-page report, *Chances and Risks of Genetic Engineering*, was approved by 16 of the 17 members of the inquiry commission ('Enquete Kommission'), composed of eight experts from industry and science and nine members of parliament (four Christian Democrats, three Social Democrats, one Liberal and one Green). Only the representative of the Greens voted against the report as an example of 'genetic imperialism'.

The report, the result of 55 meetings and 18 hearings, is designed to help the Bundestag to devise new legislation and to inform the public. It applauds the exploitation of genes and the opportunities for more efficient, economic and ecologically beneficial farming. But production of herbicide-resistant plants should be allowed only if the "use is promoted of herbicides that are without ecological or toxicological risk".

The report is not opposed to the application of genetic engineering methods in animal breeding and recommends the "promotion of the use of transgenic animals in biomedical basic research".

More reliable and precise prenatal diagnosis by means of genetic techniques are "welcomed" in the report. But there

should be a guarantee that no unacceptable abortion practices will emerge. The "sociological compulsion of abortions of embryos with genetic defects" has to be opposed.

But the screening of newborn babies to recognize treatable diseases is a "worthy instrument of preventive medicine", although screening for untreatable diseases was rejected.

The injection of genetic material into human cells is seen as a "basically acceptable form of therapy", but only if the individual is properly informed by a neutral physician or scientist and if the practice is approved by a special 'committee for biological safety' at the Bundesgesundheitsamt in Berlin.

Experiments on human germ-line cells are to be prohibited even if they are aimed at therapy, "if the germ-line cells can grow into complete human individuals".

The "guidelines for protection from dangers through *in vitro* recombined nucleic acids", created for use in gene laboratories, must be adopted in all production processes, in particular genetic work with retroviruses, cell fusion and hybrid cells. The release of genetically transformed microorganisms must be stopped for five years and this moratorium should be used for risk analysis and further research into possible ecological dangers. The release of viruses into the environment is prohibited with the exception of vaccines for humans and animals.

Generally, the commission emphasized that the existing guidelines (see *Nature* 317, 469; 1985) have proved efficient even though they are voluntary, but they should be made legally binding.

Only the Greens opposed the use of the new techniques. The commission agreed that their dissenting opinion should be added to the report. The Greens warned of the "ability of humans to manipulate humans" and rejected the placing of "resources of nature in private hands".

Jürgen Neffe

US weather service makes threat of El Niño official at last

Washington

ALTHOUGH some meteorologists and oceanographers have already declared it so, the US National Weather Service has made it official; an El Niño–Southern Oscillation (ENSO) event is under way.

In a statement on 20 January, the National Weather Service's Climate Analysis Center (CAC) said that December atmospheric and oceanic data from the equatorial Pacific "resemble those of an ENSO warming". CAC's Vernon Kousky says that the weather service delayed calling the event an ENSO episode in part because the expected inversion in the barometric pressures between the island of Tahiti and the city of Darwin in northern Australia had not occurred. But, in December, Tahiti pressure got "quite low", and the Darwin pressure was "quite high", Kousky says, suggesting that the expected seesaw of the Southern Oscillation was indeed taking place.

CAC's model of general ocean circulation indicates that westerly winds in the western equatorial Pacific at the end of 1987 have pushed surface water to the west. This should result in warming of sea-surface temperatures along the coast of Peru. Oceanographic data received by Francisco Chavez of Duke University from piers at Paita, Peru, suggest that the warm water has not yet arrived. But Chavez says all predictions are that it will make its appearance "any time now".

If there has been caution about declaring an El Niño to be under way, it is moti-

vated in part by the hysteria that has accompanied past announcements. The ENSO event in 1982–83, by all accounts much stronger than the present episode, was blamed for extreme weather conditions and millions of dollars in damage.

Kousky's estimates for the impact of the current event are much less severe. He says wetter than normal conditions along the coast of the Gulf of Mexico can be blamed on El Niño, as can be unseasonably warm temperatures in the northern sections of the United States. He adds that northern Australia and southern Africa have been somewhat drier than normal, while eastern equatorial Africa is wetter. By later this month, the warm coastal waters should bring heavy rains to northern Peru. Chavez says rainfall in the region is already slightly above normal.

Warm sea-surface temperatures and wind patterns in the equatorial Pacific have convinced many that an ENSO event was either taking place or imminent (see *Nature* 324, 504; 1986). Tim Barnett of Scripps Institute of Oceanography says Pacific sea-surface temperatures should decline slowly over the next six months and that even if this year's event is smaller than that in 1982–83, it is still noteworthy. Many were convinced as long ago as the end of last summer that an ENSO episode would occur. Barnett believes that models must take into account phenomena of low frequency — of the order of two to seven years — to be successful.

Joseph Palca

Italy participates in new telescope

THE mysterious fourth partner in the twin 8-m telescope being developed by University of Arizona, Ohio State University and the University of Chicago has been revealed. It is the Italian astronomical community, represented by the Arcetri Astrophysical Observatory in Florence. The Italians will become a full partner in the project. Rather than a cash payment, the Italian contribution will be the fabrication of the telescope, including the mounting, drive and mirror support structure. The binocular telescope, now called the Columbus Project, expects to have first light in 1992, the five hundredth anniversary of Columbus's voyage to the New Land.

Joseph Palca

New radon cancer hazard study no cause for alarm in Britain

London

MORE than 20,000 homes in the south-west of England are to be the subject of a major health study to determine how cancers caused by the emission of radon gas from their foundations can be prevented.

Radon-222, a naturally occurring radioactive gas emitted from igneous rock with uranium content such as granite, can easily be dispersed in the open air, but in the long term produces lung cancers among those living on it. Radon risks have recently been an issue in the Appalachians.

The government initiative is the result of recommendations made to the Department of the Environment by the National Radiological Protection Board (NRPB) which has studied the danger in the south-west of England, the Pennines and the highlands of Scotland. A two-year research programme on remedial and preventive measures is to be undertaken by the Building Research Establishment.

NRPB, which has previously studied the problem in mines, has recently completed a survey of 3,000 dwellings across the United Kingdom. The measurements were taken "in more detail in those areas where high concentrations would be expected to occur". The board is cautious about the dangers; it claims that "there is no direct evidence that this [lung cancer] occurs in the home, but the risk may be estimated from health studies of miners who were exposed at work".

Cornwall and Devon are the two most

reduce the level of exposure; the higher levels require immediate and "severe action". New dwellings, the board recommends, should be built to a specification that will limit such radon exposure to 5 millisieverts a year.

NRPB does not believe it is being alarmist. Apart from its studies among miners, the epidemiological evidence on lung cancer incidents among underground workers, amassed over the past 35 years, supports its view. The evidence is overwhelming from uranium miners in Colorado, Bohemia, Canada and Sweden.

Using these data, it is easy to "estimate

the risk from exposure to radon in the home over a lifetime".

The best estimate is a 2 per cent risk of dying early from lung cancer through lifetime household exposure to a dose of 20 millisieverts a year. DOE is at pains to prevent panic. Environment minister William Waldegrave claimed: "I want to stress that the risks from radon are assessed in terms of life-time exposure. There is therefore no need for drastic immediate measures to reduce levels. It is a matter of record that, in Devon and Cornwall, where radon levels tend to be higher than average, the death rate from lung cancer is lower than in many other parts of the country. The first step is to obtain an accurate measurement of the situation, so that the need for any remedial measures can be properly assessed."

Bill Johnstone

Cameroon lake gas disaster a lesson for the future

Washington

A CLOUD of 1.0 km³ of carbon dioxide gas released from Lake Nyos in Cameroon last August caused the deaths by asphyxiation of at least 1,700 people, according to a report prepared by a US scientific team that investigated the natural disaster. The US report agrees substantially with that of a UK team published earlier this year (see *Nature* 325, 105; 1987), but a key difference in what caused the gas release may determine whether simple measures can prevent similar disasters.

The US Agency for International Development sponsored the US investigation. A ten-member scientific team, drawn from universities and government laboratories, visited Cameroon in early September. Although the lake still contained large amounts of dissolved CO₂, the US team could find no evidence of excess sulphur or chlorine compounds that would be expected to accompany a large, rapid input of volcanic gas.

The team also failed to detect warming of water at the lake bottom that might reasonably accompany volcanic activity. Instead, they conclude that the CO₂ entered the lake slowly, and had a magmatic origin. Released deep in the Earth's crust, the gas lost its reactive components such as sulphur and chlorine compounds before it reached the surface. A biogenic origin for the gas is ruled out by measurements of carbon-14, the stable carbon-isotope composition of CO₂ and helium isotopes.

The US report concludes that water density differences kept the water charged with CO₂ on the bottom of Lake Nyos until something disturbed it. Edward Koenigsberg, leader of the US team, says a variety of events could have triggered the upwelling of CO₂-rich waters. Among the triggers mentioned are some seismic

event, a small rockslide from the cliffs on the lakeside or even a storm that took place earlier on the day of the disaster.

Koenigsberg says the lakes in the region are at their most unstable in August, when temperature differences between surface and bottom waters are the smallest.

Pathology reports are consistent with the CO₂ cloud explanation for the disaster. Survivors reported that victims appeared to die without a struggle. Investigations revealed no evidence for H₂S gas, although many reported smelling rotten eggs or gunpowder. The team concluded that these were olfactory hallucinations, previously reported to follow acute exposure to CO₂.

S.J. Freeth, one of the three-man British team, is sceptical about the hallucination explanation. Freeth agrees with the US report's theory of the origin of the gas, but concludes that the event was triggered by a pulse of gas coming up through a volcanic vent in the north-east corner of the lake. Such an injection of CO₂ would still possess enough H₂S and SO₂ to make their characteristic odours detectable.

A large amount of CO₂ remains dissolved in Lake Nyos, and the possibility of another fatal release remains. If the slow-release theory for the build up of CO₂ is correct, the most practical way to reduce the gas content of the lake in a controlled fashion would be to pipe water from the bottom to the surface. The US team reckons CO₂ expansion from the upward movement of water will create sufficient lift to propel the water upward without external pumping. A 9.5-cm pipe would completely 'treat' the lake in three years. But Koenigsberg says that if the pulse injection theory is accurate, such simple measures for reducing the danger will be to no avail.

Joseph Palca

On the plus side, of course,
it'll be Radon-free...



vulnerable counties in the United Kingdom although the incidence of lung cancer there is lower than in many other parts of the country, mainly large conurbations.

The NRPB survey estimated that the occupants of 20,000 homes are exposed to an unacceptably high concentration of the gas — 20 millisieverts per year. Another 2,000 homes are thought to have concentrations of two and a half times that dosage.

The lower limit is defined by the protection board as the 'action level' at which simple remedies must be implemented to

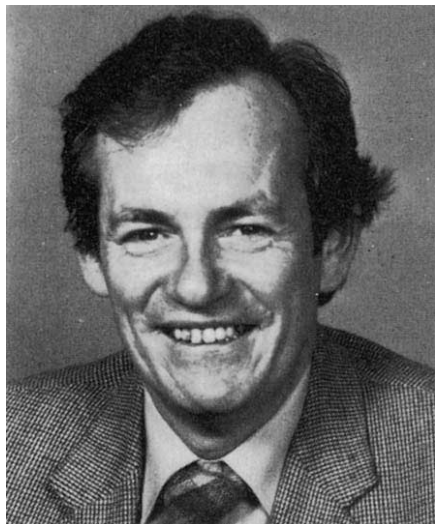
Chemist expected to head UK medical research council

London

DAVID (Dai) Rees seems set to succeed Sir James Gowans as secretary of the UK Medical Research Council (MRC). When the council meets on 5 February, it is expected formally to recommend the appointment to the Secretary of State for Education and Science, Kenneth Baker.

A chemist by training, 50-year-old Rees spent 14 years after graduating in academic life before joining Unilever Research in Colworth, Bedfordshire, in 1970. In 1980, while chairman of the Science Policy Group for Unilever, he became associate director of the MRC Cell Biophysics Unit, spending one day a week assisting the director. Two years later, Rees left Unilever to become director of the MRC's National Institute for Medical Research at Mill Hill, London.

In his four years there, Rees has organized the many separate laboratories within the institute into four groups,



From carbohydrate research to MRC secretary? bringing in Peter Rigby to head one of them and to try and infuse the whole institute with modern biological methodology. He has also initiated a Centre for Collaborative Research, of which he is acting head, dedicated to joint projects between MRC and UK-based industry. Glaxo, Wellcome, Thorn-EMI and Amersham are among the first companies to have made use of the centre.

Although more protected than most of the research councils from the government's cuts in public spending, MRC is still cash-starved and will need more industrial funds if it is to protect its own establishments, let alone expand. From that point of view, Rees's experience and continued contacts with industry have always made him a strong contender to take over the council since Gowans announced his intention to leave. That, plus his repu-

tation as a good manager, seem to have been enough to outweigh the fact that Rees, unlike some other strong contenders for the job, has no medical qualifications. His own research has veered from carbohydrate chemistry towards the chemistry and biology of cell surface interactions.

Peter Newmark

• A year after endorsing the idea of a merger between the two major UK

centres for clinical research, MRC has appointed a firm of management consultants to help decide how to proceed. The firm of Deloitte Haskins and Sells has until May to report to the Clinical Research Coordinating Committee of the council, chaired by Sir Robin Nicholson. Among its tasks is to decide which of the two existing centres, the council's Clinical Research Centre at Northwick Park or the Royal Postgraduate Medical School in Hammersmith, both in London, should absorb the other to form a cost-effective multispeciality centre of postgraduate medical research and education. □

Electronic SOS to Gorbachev shows Soviets softening on refusniks?

Rehovot

THE Soviets seem to be changing their public response to refusnik pressure. The participants in last week's meeting of the International Federation of Scientists for Soviet refusniks in Vienna expected and received support from the western delegations at the European Security Conference but surprisingly, the Soviets also accepted. They made no attempt to claim the pressure exerted on behalf of the refusniks was "interference in Soviet internal affairs".

Weizmann Institute physicist Harry Lipkin, a member of the Israeli group in Vienna, is still not sure whether "it reflects a real change in policy or the fact that Mikhail Gorbachev understands, and practises, western-style public relations". Meanwhile, in any case, Lipkin and his colleagues are determined to continue putting pressure on the Soviets, using their status and connections as scientists.

A couple of weeks ago, Soviet immigrant scientists in Jerusalem received a call for help from Alexander Ioffe, an eminent refusnik mathematician in Moscow, who had gone on hunger strike to protest at the Soviet authorities' refusal to allow his son's family to leave for Israel after earlier refusing a similar request from Ioffe himself (see *Nature* 325, 289; 1987). Ioffe asked that appeals on his behalf go to Gorbachev, Professor Guri Ivanovich Marchuk, president of the Soviet Academy of Sciences and Professor Ludvig Dimitrievich Fadeev, a Leningrad scholar who is president of the International Union of Mathematicians. Ioffe's message was translated into English by his friends in Jerusalem and transmitted by telephone to Lipkin in Rehovot, who then sent it via his computer by electronic mail to friends and colleagues around the world. They, in turn, cabled Gorbachev, Marchuk and Fadeev. Within 48 hours of Ioffe's call from Moscow, Soviet officials were being petitioned on his behalf by scores of leading scientists and scientific

bodies in the western world.

Ioffe ended his hunger strike when he was promised that his son's emigration request would be given favourable consideration and that his own request would also be reconsidered.

Weizmann Institute biophysicist Edward Trifonov was reunited with Professor Nikolai Bochkov of the USSR Academy of Medical Sciences on the Rehovot campus. Trifonov had begun his scientific career in Moscow and had once served on academic committees with Bochkov, who had come to Israel as a representative of his country's 'peace movement'.

Like Lipkin, Trifonov sees some positive changes in Soviet policies, including



the release from internal exile of his friend and mentor Andrei Sakharov (with whom Lipkin also has close ties). But knowing his Russian history, Trifonov is afraid that, as in the past, a period of democratization will be followed by a period of repression.

"So while Gorbachev may have good intentions", Trifonov concludes, "I don't trust the system".

Nechemia Meyers

Growth of US defence spending squeezes space science plans

Washington

LAST week, exactly a year after the space shuttle Challenger exploded in flight, the new US presidential science adviser, Dr William Graham, briefed a committee of the House of Representatives on the administration's plans for science, space, and technology. And once again, military objectives, not science policy, dominated the picture.

The statement from Graham, weapons expert and former acting administrator of the National Aeronautics and Space Administration (NASA), echoed the rhetoric of the Reagan administration on using science and technology to strengthen economic competitiveness while maintaining national security. He cited the 13 per cent increase in the 1988 budget for research and development and a 17 per cent increase for basic research funding through the National Science Foundation (NSF) as proof of the president's commitment to a national scientific enterprise. But when committee members probed him on specifics, Graham revealed imbalances between this administration's civilian and military science policy and his own unfamiliarity with issues outside space and defence.

The budget for 1988 includes \$64,000 million for research and development, but only \$18,200 million of that goes to civilian science and technology. Asked to explain how such a split will aid economic competitiveness, Graham replied that defence

must receive the lion's share because it requires so much more hardware. Much of this hardware is the fleet of launch vehicles planned for carrying prototype equipment for the Strategic Defense Initiative (SDI) and other military payloads.

George Brown (Democrat, California) pressed Graham to reconcile the administration's commitment to civilian space technology with the president's refusal to fund advanced space telecommunications. Graham reiterated the administration's view that government should fund "underlying" technologies and leave specific commercial applications to the private sector. The committee "does not agree with this logic", said Brown.

Committee members who were sympathetic to the administration's emphasis on space and defence displayed their own dissatisfaction with the administration's goals. As the development of SDI proceeds, they asked, will its technologies provide an infrastructure for other, non-military applications? And what will be the purpose of the much-vaunted space station, other than as a floating laboratory for experiments in strategic defence? Graham admitted that the administration could do a better job articulating its policy, but declined to do so.

The committee will submit written questions to Graham, which will give him time to consult his advisers and perhaps provide more complete answers than he did at the hearing.

Nancy Heneson

India's ballistic missile base is torpedoed by local objectors

New Delhi

INDIA's plan to establish a thousand million dollar missile base at Baliapal on the eastern coast of Orissa state has run into trouble because some 41,000 residents have refused to be evicted. They have virtually sealed all entry points with road blocks and sign boards. No civilian or defence official, not even the chief minister of the state, has been able to set foot in the area, despite an appeal from the Prime Minister, Mr Rajiv Gandhi, saying that the facility "is of overwhelming national importance".

For logistical, technical, safety and meteorological reasons, the Defence Ministry says Baliapal is the only suitable site for the base, to be known as the National Test Range (NTR), which is expected to become operational by 1990. It would launch ballistic missiles (now in an advanced stage of development at defence laboratories) and satellites in polar orbit and

would also be used for practice firing of long-range missiles, dynamic testing of electronic warfare equipment and the launching of pilotless aircraft.

According to the Defence Ministry, the shallow sea and crescent-shaped 30-km-long coastline provide a clear firing range, and nearby hills serve as observation points, making Baliapal an ideal location. According to Dr V. S. Arunachalam, the Defence Science Adviser, the Baliapal range is vital for national security. He says that none of the other 30 sites surveyed had the advantages that Baliapal offers.

Baliapal is also said to be a safe distance away from shipping lanes, aviation routes and international boundaries. The Indian Space Research Organisation says Baliapal is more suitable for the launching of its heavy remote-sensing satellites into polar orbit than is the existing launch pad at Sriharikota, which is used for putting satellites into equatorial orbit.

US AIDS education

J. Palca

IN an apparent attempt to dispel reports of feuding between their agencies (see *Nature* 325, 287; 1987) over how and when to teach children about AIDS (acquired immune deficiency syndrome), Surgeon General C. Everett Koop and Education Secretary William Bennett have issued a joint statement on AIDS education. In a concession to Bennett's concern about education without values, the statement says that just teaching children about "safe sex" will be "at best ineffectual" and at worst seriously harmful. The joint statement says AIDS education should stress "monogamy in marriage as a desirable and worthy thing", and that young people deserve the best scientific information about AIDS. □

West Germany's election

J. Neff

LAST week's general election in West Germany brought significant changes for the individual parties, although the Christian Democratic/Liberal coalition will continue to operate. The Social Democrats commanded 37 per cent of the votes, compared with 38.2 in 1983. The Christian Democrats received 44.3 per cent of the votes, their worst result since 1949, when the first elections took place in the federal republic.

The clear winners were the smaller parties: the liberals, who are seen as shifting the general political mood to more moderate attitudes, got 9.1 per cent of the votes and the Greens achieved the biggest gains, with 8.3 per cent. The coalition will continue, but with a weaker mandate. It is not clear whether Heinz Riesenhuber (CDU), the Minister of Research and Technology, will hold his post. There are rumours that he will go to the Defence Ministry and that Christian Democratic colleague Stavenhagen will get his post. The future of the Minister for Environment and Reactor Safety, Walter Wallmann (CDU), whose ministry was created after the catastrophe at Chernobyl, is said to be in doubt. □

The Defence Ministry had originally intended to acquire 130 villages in the Baliapal/Bogra area, spread over 400 km². Following popular protest, it has reduced its demand to 102 km² covering 54 villages which contribute more than \$50 million a year to the state's economy from a rich harvest of rice, fish, coconut, groundnut, cashew and betel leaves. The proposed military project would devour 50,000 acres of fertile land producing 30,000 tonnes of rice as well as 30,000 betel vines. About 1,500 fishermen and their families would be left without a livelihood.

The central government is showing great restraint in the face of a concerted campaign from the local population. It is aware that a forcible takeover of the villages might lead to bloodshed and a setback for the ruling party. K.S. Jayaraman

Plight of British postdocs

SIR—The recent report on the plight of older postdoctoral scientists in the United Kingdom (*Nature* 323, 8; 1986) will come as no surprise to most university researchers. The reasons for the discrimination against older (over 30) postdocs are quite straightforward. First, it can be more than 30 per cent more expensive to employ postdocs in their early 30s compared to newly qualified PhDs in their mid-20s. This is a real problem for academics who may wish or even prefer to employ a more experienced and mature postdoctoral assistant. It is difficult to justify this extra salary to the research councils; it is now common to be told when a grant is reviewed that only a postdoc at the lower end of the salary scale should be employed.

Second, older postdocs are less likely to remain for the full three-year tenure of most research council grants. The older a postdoc, the more experience he (or she) will have and the more anxious he will be to find a more secure job, or even to emigrate. This is especially true because he realizes that the older he is, the less employable he will be as a postdoc. The pragmatic academic will therefore tend to be biased towards the selection of more youthful applicants for postdoctoral positions.

Like many scientists of my generation, I found myself obliged to take on a series of short-term postdoctoral contracts of between one and three years' duration. During this time, I spent three years in the United Kingdom, one year each in West Germany and Australia and three years in the United States. These moves were not made out of any particular wanderlust on my part but were made necessary as each short-term contract expired.

One obvious solution is to abolish age-related salary scales for postdocs. In the United States there are no such scales. Salaries vary from university to university, and postdocs in their 40s are often paid the same salary (and do the same job) as those in their 20s. In West Germany, the equivalent of postdocs (*wissenschaftliche Assistenten*) are not on an age-related scale. While such a measure would remove the postdoc age anomaly, it would probably not entirely halt the increasing drain of scientific talent away from these shores. However, the brain drain occurs at all levels, from graduate students to professors, and is mostly due to the greater scientific and pecuniary opportunities that exist abroad.

The recent reductions in the numbers and quality of new postdocs coming onto the market, coupled with a virtual cessation of recruitment for lectureships, has produced a large 'bulge' of postdocs in their 30s. This represents a formidable

pool of talent for the nation that ought not to be allowed to go to waste. Many of us would be only too happy to employ older postdocs but find our hands tied by the present rigid and outmoded salary scale.

DENIS J. MURPHY

*University of Durham,
Department of Botany,
Science Laboratories,
South Road, Durham DH1 3LE, UK*

Primitive myth

SIR—It is a myth that preindustrial civilizations lived in harmony with nature, Jared Diamond writes in *News and Views*¹. His argument is unassailable. But by dwelling exclusively on instances of bad natural resource management, he runs the risk of perpetrating a countermyth that sound environmental practices were absent in such civilizations.

Diamond draws a number of his examples of ecological mismanagement from the islands of Oceania. What he does not mention, however, is the fact that Pacific islanders were far ahead of Western industrial civilizations in some forms of environmental management. For example, they devised and practised a wide range of marine fisheries conservation measures long before they were first used in European civilizations.

Closed seasons, closed areas, size restrictions and limited entry have been widely employed in Polynesia and Micronesia for centuries as deliberate means of preventing overfishing; the need for conservation of marine fish stocks was first recognized in Great Britain little more than 80 years ago².

To be sure, various instances of marine environmental misuse have also been documented in Pacific island cultures³. But the existence of the latter does not diminish the significance of the former. The coexistence of wise and unsound environmental practices is a characteristic of many if not all civilizations.

The danger of overemphasizing the weaknesses of environmental management in preindustrial cultures is that their strengths will be ignored. This issue is not one of mere historical accuracy. Unique environmental knowledge and time-tested environmental management practices can still be found in some of these cultures, especially in the tropics where western scientific knowledge of natural resources is weakest.

Both UNESCO and IUCN have recognized the value of traditional knowledge and management of natural resources as a complement to existing scientific knowledge. Both organizations have recently set up working groups specifically to investigate its contemporary value. The re-

cording and evaluation of such information is an urgent matter. It is disappearing rapidly as a result of the impact on native cultures of Western civilization.

R.E. JOHANNES

*CSIRO Division of Fisheries Research,
Box 1538, Hobart,
Tasmania 7001, Australia*

1. Diamond, J.M. *Nature* 324, 19 (1986).

2. Johannes, R.E. *A. Rev. Ecol. Syst.* 9, 349 (1978).

Art of citefaction

SIR—In a recent leading article (*Nature* 324, 95; 1986) you mention the possibility of citation counts being used to measure the research effectiveness of a university. Citations counts are important for other purposes and will appear to politicians to be a natural choice for this purpose, so they may well come to be used. But one consequence of this would be a considerable increase in citefaction — working to increase one's citesum — rather than research.

As in some other respects, UK academics are amateurs in citefaction. Many work in areas such as arts, basic science or engineering instead of in biochemistry or medicine where there are so many more active authors in the world that citesums are naturally much greater. Some even collaborate with industry in work that may not be publishable. Papers get published in journals with a low 'impact factor' (most UK ones). Too many try to keep references to a minimum by lazily using review articles 'where earlier references may be found' instead of listing them in full.

Citations are of two kinds. When an author references his own work, or that of a colleague, this is an 'incitation'. Incitation is largely within the control of the group and, if done systematically, can increase quadratically with the number of publications of the group whereas 'excitation' tends to increase linearly . . .

If the vice-chancellors are serious in using these counts they will need to solve some complicated issues. Citations of papers can behave quite differently. Some peak quickly but others rise slowly over a long period to great heights. One, last year, reached over 6,000 citations after a steady rise through 15 years. The citations of left, retired or deceased members of staff also pose problems of counting and allocation. Einstein last year had more than 800 citations. How are future citesums of recent work to be counted?

If citefaction and stupefaction are to be avoided, every academic should be ready to argue strongly against every suggestion that university citesums have any significance for evaluation.

G.G. HALL

*Division of Molecular Engineering,
Graduate School of Engineering,
Kyoto University, Kyoto, Japan*

Collaboration on accelerators

The US administration's backing for the newest accelerator will encourage high-energy physicists; it should also prompt them to ask what happens next in their relations with their colleagues elsewhere.

NOT every day of the week, in these times, is there news of the construction of a new particle accelerator, which is one reason why last week's announcement that the US administration will throw its weight behind the Superconducting Super-Collider (SSC) is memorable. It is easy to forget how much time has passed since the high-energy physics community began working for the proposal five years ago. And although some may prefer to use the word "lobbying", that would unfairly slight the way an awareness of costs has forced on high-energy physics an untraditional sense of responsibility.

Given the measured pace at which these projects are now designed and then financed, it could even be that SSC will be the most powerful accelerator to be put into service before this century is over. With luck, it may be working by, say, 1993, but that will not leave time for the accelerator next in line to be designed in the United States and then built before the end of the 1990s. European accelerator builders will also have their hands full until 1993 or thereabouts with the project to convert the magnet of the electron-accelerator LEP (not yet in service) to a superconducting magnet. The Soviet Union seems not to be in a mood for joining the competition at this stage, although that could change.

SSC is more than just another accelerator, both as an emblem of something and for what it will, or may, accomplish. In round numbers, it will increase by two orders of magnitude the mass of the particles that may be created artificially above that of the heavy bosons mediating the weak interaction, the Z and $W^\pm$ particles produced at Geneva, at the CERN high-energy physics laboratory, in the past two years.

On this occasion, moreover, there is more to say than the usual "so what?". At least to outsiders, particle physics is in a curious condition, littered with undiscovered particles. The undiscovered object called the Higgs particle is a continuing offence against the successful electroweak theory that accounts for the weak nuclear force, mediated by the Z and the $W^\pm$; no doubt the theoreticians are right to say that they have no idea what the mass should be, and therefore have no cause, as yet, to be downcast about their theory, but it must surely allow their imaginations to go begging for a foundation while this particle is missing.

The same (or similar) people seem to have learned to live more easily with the lack of a top quark, one of the ingredients of hadronic (strongly interacting) matter believed essential just a few years ago. The lack (so far) of a magnetic monopole and the failure (so far) to show that even protons decay, both inferences from the Grand Unified Theories fashionable early in this decade, matter less to people's pride now that attention has turned to radically different kinds of theories. In short, there are a few immediate questions that should be answered quickly when SSC is operating, but the list is not so long that the experimental programme will be determined in advance for several years.

None of this should be surprising. If one adds to the length of time taken to build a big new accelerator that required to plan it and then to win government support, the half-life for building a machine is lengthening while that of a fashionable theory is, on the average, more or less what it has always been — less than a decade, but not much less. On the face of things, if that were how events are determined, the case for building the next accelerator would be stronger with each succeeding generation. But theoreticians do learn from experiments, with the result that the two timescales are in reality tied together, which is why the strongest case for building the next accelerator is always that it will help to make theoreticians' imaginations function in a well-grounded fashion. Others, vicariously perhaps, are also enlivened in the process.

The High-Energy Physics Advisory Panel (HEPAP), advisory to the US Department of Energy, will naturally avoid notions as vague as these when seeking to justify the concepts to the US Congress. But, in reality, these forms of words may not be as inept as they seem. Why not let the Congress sense, just for once, that an essential part of the process of asking meaningful questions about the way that matter is constructed, together with the laws of nature that go with matter, is the stimulation of some factual conundrum?

Two other questions will arise in the months ahead, of which one (in this year of competitiveness) will be that of the commercial benefits to be won from building SSC; HEPAP has a sound record of telling the truth whenever possible, but on this occasion it will also be able to boast of planning to build the largest cryogenic facility (or some word like that) on the

face of the Earth, not to mention the world's most powerful accelerator. Adding up the field energy of the world's helium-cooled superconducting magnets installed in diagnostic NMR machines would not tell quite the same tale.

The more durable case for SSC is that it is technically as clever an advance on its predecessors as are most technically advanced products. SSC may not (as yet) have the cleverness of the CERN technique for making protons and antiprotons circulate in the same ring, but that or its like could yet emerge. Meanwhile, with the intention is to circulate bunches of protons in opposite directions in separate vacuum tubes within the poles of the same superconducting niobium magnets in such a way that the circulating frequency exactly matches that of the increasing magnetic field throughout a whole cycle of operation. Can that count for nothing with the US Congress? And it will surely count for something that it will take as long for ordinary mortals to walk around the instrument as they can walk, with ordinary discomfort, in a day.

The more teasing question will be that of international collaboration. The US Congress will want to know when the burden it is now asked to carry will be assumed by other legislatures' taxpayers. Whatever the number turns out to be, several thousands of millions of dollars is a lot of money. For the time being, the US Department of Energy has chosen to pin the Stars and Stripes on SSC, inviting cooperation from those elsewhere who may have a few tens of millions to spend for the sake of understanding, but who want nothing in return.

HEPAP should give some thought to this perplexing question. Many of its members' closest colleagues are people who work elsewhere, many of whom have interesting things to say about the best way to go next. Naturally, given that all accelerators have some chauvinistic attributes, experimentalists are constrained in what they can accomplish.

But everybody's interest is that high-energy physics should be made international, as in a curious way it is already: a person with a good idea or, better still the design of a good experiment, will be welcome anywhere. Why not institutionalize him (or her) by asking for an extra 0.1 per cent to found a data centre, next century's ivory tower, that keeps this interesting subject on its toes?

John Maddox

Neurobiology

Two channels reduced to one

Mark Mayer

£47

Two articles on pages 522 and 525 of this issue^{1,2} raise interesting questions concerning the molecular nature of the ion channel(s) linked to excitatory amino-acid receptors. On the basis of pharmacological experiments, neurobiologists have been using selective agonists — kainic, *N*-methyl-D-aspartate (NMDA) and quisqualic acid — as probes for individual amino-acid receptor subtypes. Together with some antagonists, these agonists form the backbone of a scheme (see figure) that has become widely accepted in the past few years^{3,4}. A simple view of ion-channel activity exemplified by the nicotinic acetylcholine-receptor channel, that individual receptors are coupled to unique ion channels, seemed to be especially true for excitatory amino-acid receptors, because the conductance mechanism activated by NMDA is known to be permeable to calcium⁵ and selectively blocked by magnesium^{6,7}, whereas responses to kainate and quisqualate behaved as if caused by the opening of magnesium-insensitive ion channels permeable to Na⁺ and K⁺ but not Ca²⁺. But the recent work by Craig Jahr and Chuck Stevens¹ and by Stuart Cull-Candy and Maria Usowicz² reported in this issue now suggests that these distinct conductance mechanisms represent different functional states of the same ion channel.

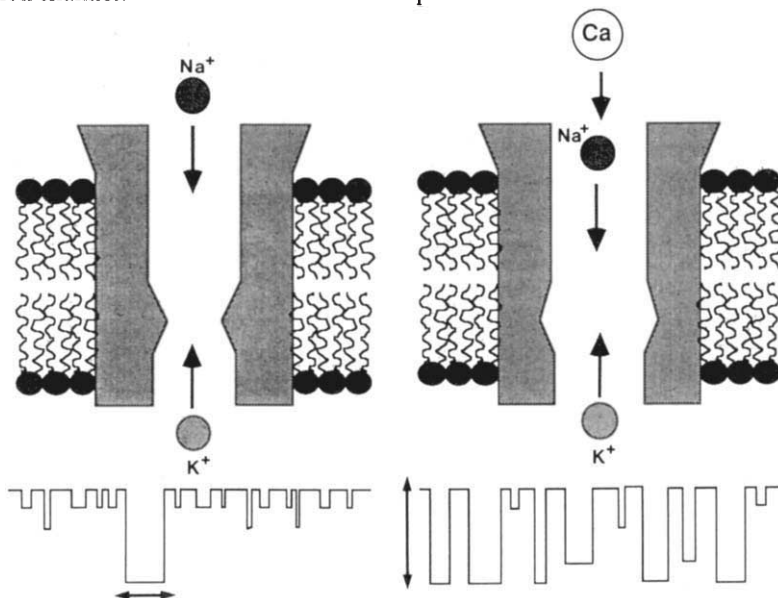
The new work, using single-channel recording from hippocampal and cerebellar neurons in cell culture, suggests that in most membrane 'patches' excitatory amino acids can activate an ion channel with multiple conductance substates, the amplitudes of which are similar for kainate, NMDA and quisqualate^{1,2}. Previously it had been shown that the ion channels opened by NMDA were of a relatively large conductance⁶ (50 pS), whereas kainate and quisqualate acted to open ion channels of much smaller conductance⁸, in the range 1–20 pS. Mixed agonists such as L-glutamate and L-aspartate were expected to activate both types of response. It now seems, however, that NMDA can also activate the small conductance channels (although infrequently) and that kainate, and especially quisqualate, can activate the large conductance channel previously thought of as 'the NMDA-receptor channel'. The selectivity of kainate, NMDA and quisqualate recorded at the macroscopic level reflects the relative rate at which these agonists cause opening of the small and large channels: for kainate and quisqualate most of the openings are to channels of small conductance, of open time approximately 0.5 ms, whereas for NMDA most openings are to the large conductance type of open time around 5 ms. This lack of an

absolute agonist selectivity is perhaps not surprising, and might reflect only the inadequacy of pharmacological probes for excitatory amino-acid receptors.

However, both Jahr and Stevens¹ and Cull-Candy and Usowicz² draw far deeper conclusions from analysis of their data. They suggest that the small and large conductance openings seen with the various amino acids reflect the activity of a single molecular complex and not the activity of two or more unique channels. Their evidence for this is good, but not yet conclusive, and depends on a statistical analysis excluding simultaneous openings of two discrete channels and the direct observation of steps between several of the sub-conductance states. A rigorous exclusion of the possibility of two or more unique channels, with conductance substates of similar amplitude, may have to wait until work similar to that of the structure-activity studies of Numa and Sakmann⁹ on the nicotinic acetylcholine receptor can be applied in this case.

Although at first glance the two papers in this issue seem remarkably similar, close inspection reveals subtle differences in the behaviour of amino-acid-activated channels in hippocampal and cerebellar neurons; whether this is genuine or reflects differences in technique is important to determine before accepting even greater complexity of excitatory amino-acid receptor channels in other areas of the central nervous system. In patches from cerebellar neurons, for example, both kainate and quisqualate activate a channel of conductance 1–2 pS in the apparent absence of any other activity. This result raises the possibility of a third molecular species: the candidates are a small conductance channel selectively activated by kainate and quisqualate receptors; and either a second 'super-channel', with multiple substates of conductance 10–50 pS, which is activated by all agonists, as proposed by Jahr and Stevens and Cull-Candy and Usowicz, or two additional channels with overlapping conductance substates, linked to NMDA and non-NMDA receptors.

Some details of the new work are highly intriguing: Cull-Candy and Usowicz report five main open states for the channel apparently activated by all amino acids, spanning the range 10–50 pS and spaced at approximately 10-pS intervals; this behaviour is reminiscent of the activity of chloride channels in epithelial cells¹⁰ and indicates that small arrays of channels somehow act together cooperatively. But direct transitions between the various open substates^{1,2}, and the higher probability of transitions from high to low conductance substates², suggest the activity of a single macromolecule. Stronger evidence for this suggestion might come from analysis of the Ca²⁺ permeability of the intermediate substates, because increasing the



Agonist-specific substates in ion channels activated by excitatory amino acids. Kainate and quisqualate are suggested to open an ion channel which is permeable to Na⁺ and K⁺ (left) and which, when activated by these agonists, opens to substates of conductance 10 or 20 pS and of lifetime around 0.5 ms (trace below left channel). Kainate and quisqualate produce occasional openings to another substate of larger conductance. NMDA is suggested^{1,2} to activate the same receptor-channel complex as kainate and quisqualate but the activity evoked by NMDA is primarily to substates of conductances 40 or 50 pS and of lifetime around 5 ms (right). These substates are permeable to Ca²⁺ as well as to Na⁺ and K⁺. Horizontal arrow, 5 ms; vertical arrow, 5 pA.

concentration of Ca^{2+} on the extracellular face of the membrane alters the reversal potential of the 50-pS channel activated by NMDA but not that of 10-pS channels activated by quisqualate¹.

The idea of a fully open channel permeable to Ca^{2+} and blocked by Mg^{2+} , with substates that exclude these ions, is intriguing. The suggestion that the NMDA antagonist phencyclidine acts as a channel blocker¹¹, but with a much slower off-rate than Mg^{2+} , also suggests some interesting experiments to test the substate hypothesis, because a channel opened by NMDA and then plugged by phencyclidine should no longer be available for activation by kainate or quisqualate receptors. Finally, modification of a superchannel, for example by phosphorylation, might link Lynch and Baudry's original hypothesis concerning the generation of long-term potentiation by favouring activation of low conductance substates at the expense of Mg^{2+} -sensitive substates normally activated by NMDA receptors, and should produce interesting general models for second-messenger modulation of amino-acid receptor channels.

Work by J. W. Johnson and Phillippe Ascher, reported on page 529 of this issue¹², demonstrates even greater complexity of excitatory amino-acid receptor channels than suggested by the above results. These experiments show potentiation of the activity of NMDA-activated ion channels by nanomolar concentrations of glycine, such that at a fixed dose of NMDA the addition of 1 μM glycine on average boosts the whole cell current 17-fold, with no action on responses to kainate or quisqualate. Single-channel recording shows this effect to occur largely by an increase in the probability of channel opening, with a small increase in mean open time and no effect on the single-channel conductance. The molecular details of the glycine binding site, its physiological significance and its relationship to the scheme discussed here^{1,2}, have yet to be determined.

The complexity of the conductance mechanisms activated by excitatory amino acids and revealed by single-channel recording is at first daunting, but perhaps

Another twist in this tale of glutamate receptors is provided by H. Sugiyama, I. Ito and C. Hirono on page 532 of this issue. They provide evidence for a quisqualate-activated receptor that seems not to be associated with an ion channel. Stimulation of this receptor expressed in *Xenopus* oocytes promotes formation of inositol 1,4,5-trisphosphate leading to an increase in intracellular free calcium. Thus glutamate joins acetylcholine and GABA as transmitters that activate both receptors associated with ion channels and those that stimulate a G-protein-mediated second messenger.

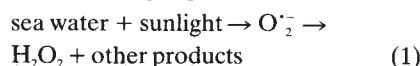
not unexpected given the recent evolution in understanding of channel behaviour from the classical mechanism of well-behaved current steps with two conformations (shut and open), to the current nightmare of models with multiple open and closed states of different conductance, connected by complex kinetic pathways with rate constants spanning many orders of magnitude — such is science. The new work described in this issue raises important questions for excitatory synaptic transmission, and clearly awaits input from molecular biology to resolve some of the controversial details. □

Marine photochemistry

Is sea water a radical solution?

Oliver C. Zafriou

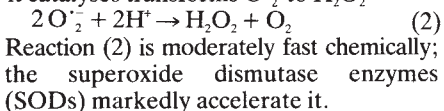
PETASNE and Zika report on page 516 of this issue¹ that photochemical reactions in sea water form two reactive, partially reduced oxygen species: superoxide anion, O_2^- ; and hydrogen peroxide, H_2O_2 .



What does this result signify? Historically, it marks a milestone in a saga of intermittent progress, much of it revealed in *Nature*. Contemporarily, scheme (1) is an integral part of a new, larger paradigm being evaluated by marine chemists.

The historical tale begins with Van Baalen and Marler's report² in 1966 of H_2O_2 in Caribbean surface waters. How does the hydrogen peroxide form? Van Baalen and Marler suggested the atmosphere, biota or unspecified photoreactions in sea water as sources. Shortly thereafter, Swallow³ reported that cosmic rays, ^{40}K decay and possibly photoionization of phenolic compounds produce hydrated electrons, e_{aq}^- , in the ocean. These e_{aq}^- rapidly reduce oxygen to O_2^- .

In 1969 McCord and Fridovich⁴ also reported an enzyme activity that formed the basis for testing scheme (1). The reaction it catalyses transforms O_2^- to H_2O_2 .



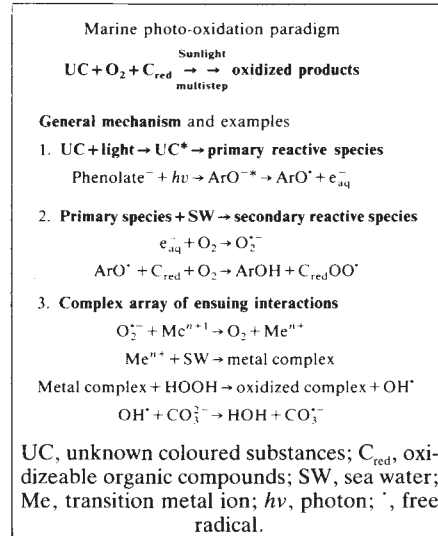
Thus, the ideas and the methods needed to formulate and evaluate scheme (1) existed in 1970, but lay fallow for years. Western marine chemists were primarily occupied with thermodynamics and notions of the biota as agents perturbing equilibrium (photosynthesis) and selectively catalysing partial relaxation towards equilibrium (the rest of the biosphere). Esoteric chemical species such as e_{aq}^- seemed curiosities. Workers in the Soviet Union⁵ were thinking more chemically, but did not test the $\text{O}_2^- \rightarrow \text{H}_2\text{O}_2$ scheme

1. Jahr, C.E. & Stevens, C.F. *Nature* **325**, 522 (1987).
2. Cull-Candy, S.G. & Usowicz, M.M. *Nature* **325**, 525 (1987).
3. Watkins, J.C. & Evans, R.H. *A. Rev. Pharmac. Tox.* **21**, 165 (1981).
4. Mayer, M.L. & Westbrook, G.L. *Prog. Neurobiol.* **28**, 197 (1987).
5. MacDermott, A.B. *et al.* *Nature* **321**, 519 (1986).
6. Nowak, L. *et al.* *Nature* **307**, 462 (1984).
7. Mayer, M.L. *et al.* *Nature* **309**, 261 (1984).
8. Ascher, P. *et al.* in *Ion Channels in Neural Membranes* (eds Ritchie, J.M., Keynes, R.D. & Bolis, L.) 283 (Liss, New York, 1986).
9. Mishina, M. *et al.* *Nature* **321**, 406 (1986).
10. Krouse, M.E. *et al.* *Nature* **318**, 58 (1986).
11. Honey, C.R. *et al.* *Neurosci. Lett.* **61**, 135 (1985).
12. Johnson, J.W. & Ascher, P. *Nature* **325**, 529 (1987).

Mark Mayer is a visiting professor in the Laboratory of Developmental Neurobiology, National Institute for Child Health and Development, NIH, Bethesda, Maryland 20892, USA.

experimentally.

By the early 1980s, efforts to evaluate scheme (1) had begun in earnest. Zika's group⁶ showed that H_2O_2 forms photochemically in sea water and that the photolysis of natural phenolic compounds yielded e_{aq}^- , and in 1983 a Canadian group⁷ reported photochemical formation of O_2^- in fresh waters. Their results should have



applied to coastal sea water as well, thus essentially validating scheme (1) — Swallow's suggestion³ plus reaction (2). Alas, by 1984 the synthesis came unravelled (along with quite a few biochemical papers) when the technique used for superoxide was found "inappropriate"⁸.

Now Petasne and Zika¹, comparing H_2O_2 formation in SOD-spiked and unspiked sea water, reconfirm that O_2^- forms photochemically and decays mainly to H_2O_2 . The qualitative confirmation of scheme (1) is the essence of their contribution. They also present a quantitative analysis of the SOD effect and suggest other pathways. Given the assumptions, extrapolations and internal inconsistencies

in the kinetics, this analysis is somewhat more tenuous.

If the era of testing this scheme is closing, one of incorporating it into a larger paradigm is just beginning. The oxygen reduced to H_2O_2 must be balanced by equivalent oxidation elsewhere. But where, how and by how much? Most of the recent work related to the larger aspects of the problem^{6,9} can be accommodated by the heuristic scheme shown in the figure. This diagram adapts the classical chemical auto-oxidation/photo-oxidation mechanism to the situation in the ocean. Such pathways were elucidated for other complex reactions such as smog formation and the low-temperature or light-induced reactions of many materials containing organic components or reduced transition metals (for example, cellular lipids, foods, plastics, paints and corrodable metals) in contact with air¹⁰. Photo-oxidations characteristically involve reactive free-radical intermediates (O_2^- is one), and often involve cyclic oxidation-reduction of transition metals.

Oceanic conditions differ so markedly from those of well-studied photo-oxidation/auto-oxidation environments that applying the paradigm to the oceans, far from being straightforward, will provide a challenge. A solute molecule travelling with the water, for example, experiences more than a decade of exposure to sunlight in the upper 40 metres per global oceanic 'stir'. The interpretive challenge is perhaps illustrated by noting that the side reactions of O_2^- reported by Petasne and Zika result in reduction, not oxidation, of unknown materials. Is the paradigm fundamentally awry, or are reductions by O_2^- (and H_2O_2) mere countercurrent eddies in a larger oxidative stream? Work in marine photochemistry, long supported in the United States by the National Science Foundation, is now a "special focus program" of the Office of Naval Research. Additionally, several European scientists have begun to work in the area. Thus, we may soon learn more about oceanic photochemistry. But it is no longer a radical solution to consider the ocean as a solution of radicals. □

1. Petasne, R.G. & Zika, R.G. *Nature* **325**, 516-518 (1987).
2. Van Baalen, C. & Marler, J.E. *Nature* **211**, 951 (1966).
3. Swallow, A.J. *Nature* **222**, 369-370 (1969).
4. McCord, J.M. & Fridovich, I. *J. biol. Chem.* **25**, 6049-6055 (1969).
5. Sytchev, A. Ya., Travin, S.O., Duka, G.G. & Skurlatov, Yu.I. (ed. Batyr, D.G.) *Catalytic Reactions and the Protection of the Environment* (Shtiintsa Publishers, Kishinev, 1983).
6. Zika, R.G. & Cooper, W.J. (eds) *Photochemistry of Environmental Aquatic Systems* ACS Symp. Ser. 327 (Am. Chem. Soc., Washington DC, 1987).
7. Baxter, R.M. & Carey, J.H. *Nature* **306**, 575-576 (1983).
8. Bielski, B.H.J., Arudi, R.L., Cabelli, D.E. & Bors, W. *Analyt. Biochem.* **142**, 207-209 (1984).
9. Zafiriou, O.C., Jousot-Dubien, J., Zepp, R.G., & Zika, R.G. *Envir. Sci. Technol.* **18**, 358A-371A (1984).
10. Scott, G. *Chemistry in Britain* **21**, 648-653.

Oliver C. Zafiriou is at the Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA.

Vertebrate palaeontology

Conodonts classified at last

Michael J. Benton

CONODONTS, small tooth-like fossils found in Palaeozoic (570–245 million-year-old) rocks, were until recently a mystery. Many supposed conodont animals have been reported, but it was not until 1983 that an acceptable specimen was described¹. New findings² improve our knowledge of this conodont animal and its affinities, and a second, older, form of conodont animal has just been reported^{3,4}.

Conodonts are composed of calcium phosphate (apatite), as are the bones of vertebrates, and are useful index fossils for dating rocks. Many conodont experts have contented themselves with dissolving rock samples, extracting the conodonts, each only about 1 mm long, and using them for dating. The first crucial biological information was obtained in the 1930s with the discovery^{5,6} of a bilaterally symmetrical arrangement of three or four different types of conodonts, assumed to be some sort of feeding device. But the subsequent confirmation that conodonts all form 'feeding baskets' did not indicate what the animal was. In the past 20 years, conodonts have been described as plants, coelenterates, Aschelminthes, Gnathostomulida, molluscs^{7,8}, Tentaculata, chaetognaths and chordates.

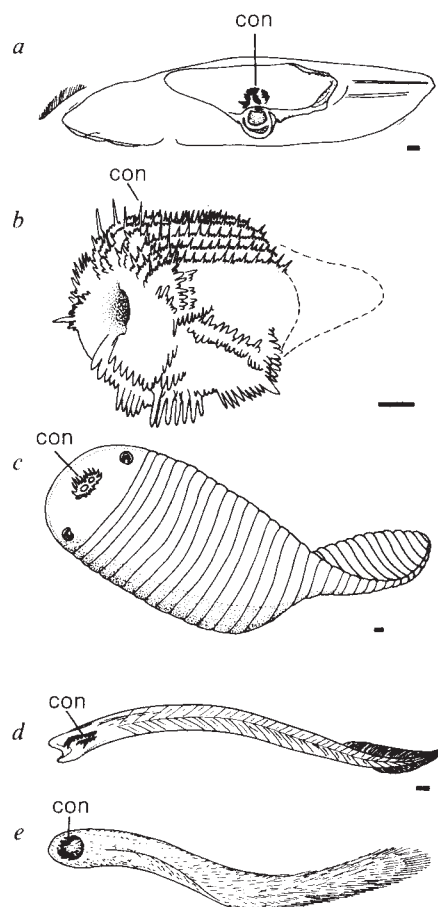
The first supposed conodont animal was described⁹ in 1973. The animals bearing the conodonts have a long flattened cigar-shaped body with a fin-like structure at the posterior end (a in the figure). There were several conodonts in the central part of the animal, which seemed to have a nerve cord near the front, and the animal was made the type of a new Phylum Conodontochochordata, related to the chordates.

Soon after the description of this animal had been published, Lindström¹⁰ and Conway Morris¹¹ suggested that it had merely ingested the conodonts, which were in the stomach region, not the head: it turned out that the guts of some specimens contained mixtures of different conodont apparatuses.

As well as debunking one conodont animal, these authors described two more. Lindström illustrated a hypothetical conodont animal in which the conodont elements faced outwards on a body shaped like a toilet roll (b in the figure). Conway Morris described, on the basis of fossils, a wide, flat animal about 6 cm long with ring-like structures around its body (c in the figure). There is an opening ringed by impressions of curved tooth-like elements in the head region underneath. This animal, named *Odontogriphus*, comes from the Middle Cambrian (about 540 million years ago) of western Canada, and

the tooth-like elements are rather like some fossils identified as ancestral to true conodonts. The preservation of *Odontogriphus* is not good enough, and the ancestry of conodonts is too uncertain, for it to be clear whether or not this is a conodont animal.

In 1983 the 'real' conodont animal was described¹ from the Carboniferous of Edinburgh and was discussed in a News and Views article¹². This 4-cm-long, worm-shaped animal had a conodont apparatus in its head region, traces of muscle blocks in the trunk and some kind of tail fin. It seemed to have affinities to both the chordates and to the chaetognaths (arrow worms), but it was placed in a separate, intermediate, Phylum Conodonta. This conodont animal has since



The conodont animal. a, A Carboniferous fossil from North America; b, a hypothetical animal with the conodonts facing outwards; c, *Odontogriphus*, a Cambrian fossil from western Canada; d, the Carboniferous fossil from Scotland interpreted as a chordate; and e, interpreted as an aplousophoran mollusc. Scale bar, 1 mm; con, conodonts. (After refs 9, 10, 11, 2 and 8, respectively; drafted by L. Lawson.)

been assigned^{7,8} to all three of these groups and also to the Mollusca.

The molluscan interpretation seems the most unusual. In general outline the Scottish conodont animal resembles living shell-less molluscs called Aplacophora. Tillier and colleagues^{7,8} believe the conodonts to be equivalent to the molluscan radula (the rasping feeding structure) because of morphology, and because both are made of calcium phosphate. The chevron-shaped 'muscle blocks' in the conodont animal are interpreted as the spicules that occur over the body surface of aplacophoran molluscs, and the tail fin is seen as the elongated spicules on the back (*e* in the figure). However, this molluscan interpretation is weakened by the new fossil discoveries² from Scotland, which reveal further anatomical details (*d* in the figure) and strengthen the view that the conodont animal was a true chordate with V-shaped muscle blocks, a laterally flattened body and unequal tail fins. In particular, the Edinburgh conodont animal has close affinities with the living myxinoidea (the jawless hagfishes), which have similar feeding apparatus consisting of several rows of teeth that can be turned

outwards for parasitic feeding. The new Silurian conodont animal^{3,4}, although less well known, appears to confirm the chordate affinities of the conodonts.

The conodonts seem to have found a home at last, and a long-standing palaeontological puzzle has been largely solved. The interesting questions now concern the exact placing of the conodonts in relation to the various primitive chordate groups, for which more well-preserved specimens will be needed. □

1. Briggs, D.E.G., Clarkson, E.N.K. & Aldridge, R.J. *Lethaia* **16**, 1 (1983).
2. Aldridge, R.J., Briggs, D.E.G., Clarkson, E.N.K. & Smith, M.P. *Lethaia* **19**, 279 (1986).
3. Mikulic, D.G., Briggs, D.E.G. & Kluesendorf, J. *Science* **228**, 715 (1985).
4. Smith, M.P., Briggs, D.E.G. & Aldridge, R.J. in *Palaeobiology of Conodonts* (ed. Aldridge, R.J.) (Horwood, Chichester, in the press).
5. Scott, H.W. *J. Paleontol.* **8**, 448 (1934).
6. Schmidt, H. *Palaeontol. Z.* **16**, 76 (1934).
7. Tillier, S. & Cuif, J.-P. *C.r. hebdomadaire. Séances. Acad. Sci., Paris* **303**, 627 (1986).
8. Tillier, S. & Janvier, P. *La Recherche* **17**, 1574 (1986).
9. Melton, W.G. & Scott, H.W. *Geol. Soc. Am. Spec. Pap.* **141**, 31 (1973).
10. Lindström, M. *Palaeontology* **17**, 729 (1974).
11. Conway Morris, S. *Palaeontology* **19**, 199 (1976).
12. Higgins, A. *Nature* **302**, 107 (1982).

Michael J. Benton is in the Department of Geology, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland.

Cultural evolution

Why do animals specialize?

William J. Sutherland

ECOLOGISTS have tended to consider all individuals within a single population as being equivalents; that is, having the same ecology and behaviour. But a closer look at any population reveals that individuals can be very different and that these differences change the dynamics or behaviour of the population as a whole. Why do individuals in the same population, and thus exposed to the same environment, exhibit different behaviours? Are the causes of these differences genetic, cultural or learnt¹? Detailed studies of the oystercatcher *Haematopus ostralegus* show that these birds exhibit a remarkable diversity of feeding specializations and their feeding behaviour has become a classic example of culturally determined behaviour in birds². Recent work helps to unravel the causes of these specializations and shows that the role of culture may have been greatly exaggerated. It may be more useful to look for ecological explanations for this specialization.

By marking birds with individual combinations of colour rings, it can be shown that an oystercatcher can feed almost entirely on one species, for example mussels or cockles, throughout the winter³.

Among the individuals that specialize on mussels some, called stabbers, push their bill between the valves of the mussel whereas others, hammerers, use violent blows of the beak against one valve until it cracks and then insert their bill through



Hammerer (above) and worm-feeder (below).

the break and chisel out the flesh³. There are even more specializations among the hammerers⁴: some birds consistently break into the dorsal side and others break into the ventral surface. Individuals may specialize further by attacking either the left-hand or the right-hand valve⁵. No doubt there are yet more undiscovered specializations.

Why should oystercatchers show such

extreme individual specializations? A long-term field study on the Exe estuary, United Kingdom, by Goss-Custard and colleagues^{4,6-8} helps to answer this question. One likely explanation is that bivalves are a difficult prey to tackle. Oystercatchers might have to exploit different weaknesses in different mussels and practise using one technique for maximum profit. Dorsally hammering oystercatchers thus pick out those mussels whose dorsal shell has worn thin from abrasion by sand carried over it by the tide. In any one patch of mussels, only a few individuals have shells thin enough for the birds to break into. The oystercatchers find these vulnerable prey by tapping the shells with the tip of their beaks⁶. Ventral hammerers also seek out mussels with thin shells, though on the ventral side. They cannot tell the thickness of the shell underneath from its thickness on the dorsal side because the two measures are unrelated, and therefore they need another cue. The colour of the shell, and perhaps the presence of barnacles, give the bird a clue⁶. Young mussels that grow fast tend to have thinner shells on the underside than do the older and slower-growing ones. The fast-growing ones are blacker and often covered in barnacles, so the birds could use the general appearance of the mussel to gauge the thickness of the shell underneath. Even so, they often make mistakes and give up trying to hammer into a significant proportion of the mussels they tear off the mussel bed⁹. The defences of the mussels mean that it pays an individual bird to stick to one method of attack.

The specialization is reflected in bill shape, which is generally correlated with the feeding technique and diet. By marking the bill, J. Hulscher¹⁰ and C. Swennen and co-workers¹¹ in Holland discovered that oystercatcher bills replace themselves once every six months. This rapid turnover of the bill allows the bill shape to adjust quite quickly to a change in feeding technique. Hulscher¹⁰ showed that when oystercatchers move from their coastal wintering areas to breeding areas inland, their bill shape changes within two weeks from the blunt shape of a hammering mussel specialist to the pointed bill of a worm specialist.

The shape that wear imposes on the bill might restrict the options for flexibility. The case for this hypothesis seems strongest when considering mussel and worm specialists. To catch a worm, the bird must force its bill tip into the ground and a pointed beak may be more efficient for this than one with a flattened tip¹¹. It is not so clear, however, that the different bill shapes attained by members of the oystercatcher community that open mussels in different

ways reinforce the specialist feeding technique of the birds.

Norton-Griffiths³ was the first to distinguish between hammering and stabbing techniques in oystercatchers. He also showed that hammering parents always produce young that hammer whereas stabbing parents produce young that stab. To distinguish whether this inheritance has a cultural or genetic basis he switched eggs between nests belonging to hammerers and stabbers. Only three chicks survived to independence, but performed the technique of the foster parents, which implies that the feeding behaviour of young birds is learnt and is not determined genetically. Adult birds pair only with another bird of the same speciality¹².

More recent studies cast doubt on this simple story. The main objection is that the birds are much more flexible in their feeding habits than was previously thought. On the Exe estuary, for example, the diet and behaviour differs between birds of different ages⁷. Most juveniles consistently stab their prey, perhaps because their bills are soft, but many change to one of the hammering techniques as they mature: marked individuals that stabbed in the first winter can be seen experimenting with hammering in later years.

There is also far more flexibility among adults in diet and feeding technique than was previously thought⁸. Birds in captivity and colour-ringed birds on the Exe regularly feed in various ways and may switch from one specialization to another¹³. Although all birds studied had a favoured method, they quite frequently used an alternative.

Although many chicks learn a technique from their parents, it seems that the generally accepted story of cultural evolution is an insufficient explanation of the specializations. As the evidence I have discussed shows, specializations are not rigidly adhered to and may change as a bird matures. Indeed, many oystercatchers are reared inland¹⁴ and many of these

individuals feed on shellfish during the winter¹⁰, so the chicks cannot learn about shellfish from their parents, which abandon them before returning to the coast.

In general terms, it seems that each diet and feeding technique has associated costs and benefits which may vary between individuals according to several factors. By taking different sections of the prey population, individuals reduce indirect competition caused by depletion of the avail-

able food supply. The best strategy for an individual could depend on its bill shape^{5,11} and bill size¹⁵, the prey population⁶, the behaviour of others⁸ and the individual's previous experience¹², but the relative importance of each component is still unknown. □

William J. Sutherland is in the School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK.

Ocean Drilling Program

News from a deepening hole

Leg 111 shipboard scientific party*

ONE of the main objectives of the Ocean Drilling Program (ODP) is to core as deeply as possible beneath the ocean floor to provide experimental evidence for models of the structure of the oceanic crust. With one exception, drilling so far has penetrated no further than 1 km through the sediments and the basaltic pillow lavas that make up the first two layers of the crust and are extruded at the mid-ocean ridges. The only borehole that clearly penetrates deeper than the pillow lava layer is hole 504B, originally drilled by the Deep Sea Drilling Project (DSDP) in the eastern equatorial Pacific. Leg 111 of ODP cored this hole as deeply as possible into the sheeted dykes and logged it with advanced borehole geophysical tools. We sought to study the alteration of the basalts, which has occurred in several stages since the crust was formed, and is controlled primarily by hydrothermal circulation through the cooling crust.

Drilling

Typically, the first two layers of the ocean crust constitute roughly 1–2 km of the 5- to 7-km-thick crustal section, so the deeper part of the oceanic crust has never been directly sampled. Much of our information about the deeper crust is derived from seismic studies and from ophiolites — slices of crust that formed at either mid-ocean ridges or back-arc spreading centres and were later tectonically emplaced sub-aerially. To produce hole 504B, three legs of the DSDP drilled to a depth of 1,350 m

beneath the sea floor in crust that formed 5.9 million years ago at the Costa Rica rift. The hole included 275 m of sediments, 575 m of pillow lavas and 200 m of transition into 300 m of the underlying layer of basaltic sheeted dykes that formed at the spreading centre by intrusion beneath the extrusive pillow lavas.

We encountered very difficult drilling conditions in the massive sheeted dykes, but increased the depth of the hole to 1,562.3 m. We recovered 26.4 m of core, or 12.4 per cent of the 212.3 m drilled — these rocks were all olivine tholeiitic basalt from massive dyke units. We sampled five intrusive dyke contacts with dips between 75° and vertical.

Most of the recovered basalt is slightly altered, being about 10–20 per cent recrystallized. The patterns of secondary minerals deposited in veins and cracks indicate that the alteration occurred in at least three stages: first, reactions with sea water at the spreading axis at temperatures of 300 °C; second, circulation of more evolved axial hydrothermal fluids, forming cross-cutting veins; and third, lower-temperature hydrothermal circulation as the crust cooled away from the axis of spreading.

A regular variation in the pattern of heat flow from the surface of the sediments in a 15-km square around hole 504B indicates that the last stage of alteration may still be occurring. We cored the 200–300 m of sediments for 5 days at local maxima and minima in heat flow within 3 km of hole 504B. Gradients in the concentrations of calcium, magnesium and sulphate ions in the pore waters of the sediments indicate that the pore fluids presently convect through the sediments at very slow rates of the order of a few millimetres per year. The pore-water chemistry also suggests that pore fluids circulate more vigorously within the uppermost basalts immediately below the sediments.

Temperatures and permeabilities in hole 504B indicate that all but the upper 100–200 m of basalts are presently im-

1. Partridge, L. & Green, P. in *Behavioural Ecology* (eds Sibly, R.M. & Smith, R.H.) 207–226 (Blackwell, Oxford, 1985).
2. Bonner, J.T. *The Evolution of Culture in Animals* (Princeton Univ. Press, 1980).
3. Norton-Griffiths, M. *Ibis* **109**, 412–424 (1967).
4. Goss-Custard, J.D. *et al. Anim. Behav.* **30**, 917–928 (1982).
5. Hulscher, J.B. *Ardea* **70**, 89–152 (1982).
6. Durell, A.E.A. le V. dit. & Goss-Custard, J.D. *Anim. Behav.* **32**, 1197–1203 (1984).
7. Goss-Custard, J.D. & Durell, S.E.A. le V. *Ibis* **125**, 155–171 (1983).
8. Goss-Custard, J.D. *et al. J. anim. Ecol.* **53**, 233–245 (1984).
9. Meire, P.M. & Erynck, A. *Anim. Behav.* **34**, 1427–1435 (1986).
10. Hulscher, J.B. *Neth. J. Zool.* **35**, 124–154 (1985).
11. Swennen, C. *et al. Neth. J. Sea Res.* **17**, 57–83 (1983).
12. Norton-Griffiths, M. thesis, Univ. Oxford (1968).
13. Goss-Custard, J.D. & Sutherland, W.J. *Anim. Behav.* **32**, 299–301 (1984).
14. Cramp, S. & Simmons, K.E.L. *Handbook of the Birds of Europe, the Middle East and North Africa*. Vol. 3 (Oxford Univ. Press, 1983).
15. Dare, P.J. & Mercer, A.J. *Bird Supply* **20**, 173–184 (1973).

*Co-chief scientists: Keir Becker (Univ. Miami) and Hitoshi Sakai (Ocean Research Institute, Univ. Tokyo). ODP Staff Scientist: Russell B. Merrill (ODP, Texas A&M Univ.). Also Andrew C. Adamson (ODP, Texas A&M Univ.), Jeffrey C. Alt (Washington Univ.), Daniel Bideau (IFREMER, France), Peter M. Herzig (Aachen Univ. of Technology), Hodaka Kawahata (Univ. Toronto), Hideo Ishizuka (Kochi Univ., Japan), John Malpas (Memorial Univ., Canada), Joel Sparks (Univ. Massachusetts), Stefan Uhlig (Univ. Giessen, FRG), Harue Masuda (Univ. Tokyo), Michael J. Mottl (Hawaii Inst. Geophysics), Joanne Alexandrovitch (Lamont-Doherty Geological Observatory), Simon Houghton (Open Univ., UK), Hajimu Kinoshita (Chiba Univ., Japan), Janet E. Pariso (Univ. Washington), Joseph Phillips (Univ. Texas at Austin), Roger N. Anderson (Lamont-Doherty Geological Observatory), Robert Gable (Bureau de Recherches Géologiques et Minières, France), Michael A. Lovell (Univ. Nottingham), Roger H. Morin (US Geological Survey, Denver) and Philippe Pezard (Lamont-Doherty Geological Observatory).

Benjamin Levich (1917–1987)

BENJAMIN Levich, the most highly placed 'refusnik' scientist to be allowed to emigrate from the Soviet Union, died on 19 January. Levich was known internationally for his work in physicochemical hydrodynamics, which he was the first to establish as a separate scientific discipline. His research activities included gas-phase collision reactions, the quantum mechanics of electron transfer and the development of a rotating-disk electrode as a tool for research.

By 1972, he had attained the post of Professor of Chemical Mechanics at Moscow State University (the chair was created especially for him), and was a corresponding member of the Soviet Academy of Sciences. But in that same year, after consultations with his wife Tanya and his sons Alexander and Evgenii, he decided to apply for emigration to Israel.

Immediately his teaching post was abolished, he was demoted to the rank of a 'scientific worker', and shortly afterwards his younger son Evgenii was illegally transported to a military camp in the Soviet Arctic. Levich was informed that he would never be allowed to emigrate, because of his knowledge of state secrets.

A campaign for the Levich family was organized by Dr Brian Spalding of Imperial College London. This campaign attracted considerable attention, particularly when it became known that western publications including *Nature*, distributed in photostat form in the Soviet Un-

ion, had all references to Levich deleted from them. But the campaign was suddenly halted with the news that Levich had reached a gentleman's agreement with the emigration authorities: if the public outcry ceased, Alexander and Evgenii would be allowed to emigrate at once together with their wives, and Benjamin and Tanya would follow a year later. But when the time came for the senior members of the Levich family to emigrate, the Soviet authorities denied any knowledge of such an agreement.

In 1977, a conference on physicochemical hydrodynamics was held in Oxford, in honour of Levich's 60th birthday. Such an honour is virtually a matter of routine for scientists of his rank in the Soviet Union, but, in his circumstances, no Soviet honours could be expected. The conference was so successful scientifically that it was decided to launch a regular series of Levich conferences. The second was held in Washington, DC in 1978, and within three weeks, Benjamin and Tanya Levich arrived in the west. They settled first in Israel, where Levich had a chair reserved for him at Tel Aviv University, and in 1979, he accepted the Albert Einstein chair of physics at New York City College, commuting regularly between the United States and Israel. Shortly after he took up this post, however, his wife Tanya had to have massive heart surgery, from which she never fully recovered. She died in 1984.

Vera Rich

permeable to such circulation, largely because the original porosity of the basalts has been sealed by deposited alteration products. The upper 100–200 m of basalts remain permeable, and in fact ocean bottom water actually flows down the hole and into this section of basement. This flow was observed when the hole was first drilled by DSDP Leg 69, but the rate of flow has decayed to less than 1 per cent of its original value.

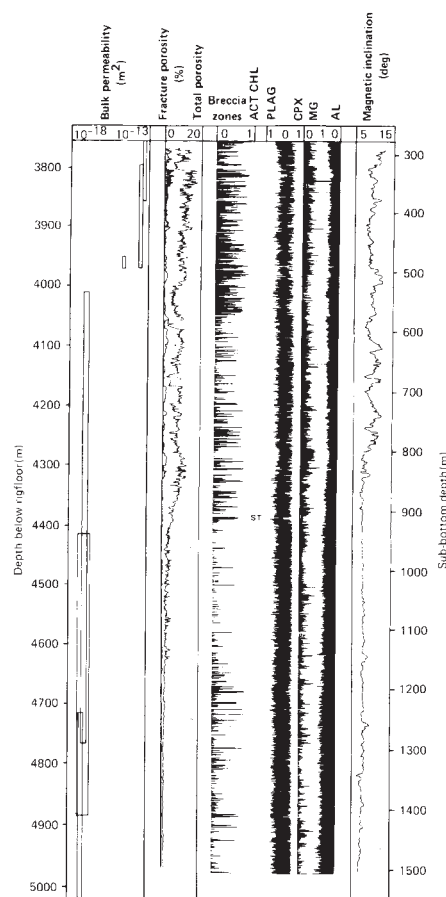
Similar downhole flow of ocean bottom water has been observed in other holes drilled through the sedimentary layer into young oceanic crust. This flow does not affect the conductive temperature profile in the deeper layers of hole 504B. The equilibrium crustal temperature at the total depth of 1,562.3 m is estimated to be 165 °C.

Logs

Given the poor recovery of core from hole 504B, our geophysical logs were especially important in providing a continuous image of the formation (see figure). Several logs, particularly those of sonic velocities and electrical resistivity, clearly distinguish individual extrusive and intrusive basaltic units that are of the order of 10–20 m thick. We also looked at

these units with sophisticated neutron-activation tools that resolve relative abundances of several key elements and allow the construction of continuous logs of geochemistry and mineralogy. These 'chemical logs' show that the secondary alteration products are confined to fractures along contacts between relatively unaltered units of fairly homogeneous geochemistry. This confirms that contacts between units were originally porous and permeable, but have been sealed by deposition of hydrothermal alteration products. The logs show that the present porosity of the sealed crust is quite low, ranging from 10–15 per cent in the upper 100–200 m of pillow lavas to less than 1 per cent in the deepest sheeted dykes.

A magnetometer log revealed a significant difference in the magnetic inclinations of the pillow lava section and the sheeted dykes. The overlying pillow lavas are inclined about 15 per cent, whereas the dykes below are inclined less, about 8 per cent. This seems to require a tectonic explanation, and suggests that the contact between pillow lavas and sheeted dykes in hole 504B is a relic of early listric faulting within the axial rift. Such faulting might have provided the highly permeable conduits for circulating hydrothermal



Geophysical experiments in hole 504B. Left to right: bulk permeability measured by the packer experiment; fracture and total porosities determined from electrical resistivities; hypothetical (normative) mineralogies; and relative magnesium oxide and aluminium oxide contents. Normative mineralogies were determined by recalculating the content of Si, Al, Fe, Mg and Ca into the normative components actinolite (ACT), chlorite (CHL), plagioclase (PLAG), clinopyroxene (CPX), olivine and smectite, assuming typical local compositions for the normative minerals. Normative plot units indicate the fraction (1 is 100 normative weight per cent) of the rock formed by each normative component. Relative contents of magnesium oxide (MG) and aluminium oxide (AL) are shown as counts, where 1 signifies the maximum observed. Average amount of magnesium oxide is 7 weight per cent and that of aluminium oxide is 22 weight per cent. ST denotes the 18-m-thick stockwork-like unit.

fluids that produced an 18-m-thick mineralized stockwork sampled at the base of the pillow lavas.

Our detailed vertical seismic profile of the hole showed that there are two major seismic reflectors about 100 and 450 m deeper than the hole now extends. One of these reflectors is probably the transition between the sheeted dykes and the ultramafic gabbros, the next major layer of the oceanic crust. This transition may be within reach of the next visit to hole 504B.

Forest management

Burning issues in fire control

Peter D. Moore

THE use of fire for forest management may seem a strange concept to those accustomed to the extensive fire breaks, warnings and precautions usually associated with commercial forests in northern Europe. But fire is now a regular feature of forestry in North America and is being proposed¹ as a means of efficient management for the dry forests of South-East Asia.

In the 1960s it was estimated that an average of 4.8 million acres of forest in the United States was burned each year as a result of accidental fires². But it was also recognized that the accumulation of dry brushwood beneath the canopy was an important factor in forest flammability, and that this build-up was itself an undesirable consequence of success in fire control. Periodic fires while the underbrush was still sparse could protect the forest from more intense and harmful fires at a later stage. So in 1967 controlled burning was carried out in 2.9 million acres of both private and government-owned forests, with the destruction of an estimated 61 million tons of brushwood. This was the culmination of 30 years of gradually altering opinion on the part of foresters in North America. The value of such prescribed burning is now sufficiently well established for its use over 10 million hectares of pine forests in the south-eastern United States alone³.

The ecological justification of prescribed burning depends to a considerable extent on historical precedent, as well as practical benefit in reduced wildfire frequency. Wright⁴ reviewed the palaeoecological evidence from charcoal in lake sediments and fire scars within the annual growth rings of trees and showed that areas of Minnesota during the past few centuries had burned fairly regularly on an approximate 100-year cycle. In the red-wood forests of the West, Weaver⁵ found that there had been at least 45 fires in the past 1,100 years and that fire suppression by white settlers was an innovation of dubious value to the forest. Similar studies in Canada⁶ show that a natural fire cycle with a wavelength of about a century was occurring a thousand years ago, but without change in the overall forest composition.

Artificial removal of the fire factor could destroy this stability and precipitate a new development in the forest succession, perhaps leading to replacement of the characteristic conifers by hardwood species. Williamson and Black⁷ carried out controlled fires in forest stands of pine (*Pinus palustris*) and two oak species (*Quercus laevis* and *Q. geminata*). By measuring temperature profiles in the dif-

ferent forests using waxes of various melting points, they found that although pine burns at almost 300 °C at ground level and achieves a maximum of 150 °C at a height of 4 m, the corresponding figures for oaks are only about 200 °C at the base of the tree and less than 100 °C at 4 m. The ecological implication is that litter fires under a pine canopy are sufficiently hot to eliminate invasive oaks. The flammability of pine litter is therefore a method of competitive exclusion of the oaks that

At 2 m, the outer bark temperatures of trees were usually less than 200 °C and most trees could cope with outer bark temperatures of 300 °C because of the insulating properties of the bark itself; inner bark temperatures rarely exceeded 75 °C.

Where litter had been allowed to accumulate to a depth of more than five or six leaves, or where ground cover of herbs, bamboos and small shrubs had built up, the burn became much more intense and temperatures of between 700 and 900 °C were recorded. Fortunately, the very low organic matter content of the soil always prevented significant penetration of the burn and just 5 cm below the surface the temperature was usually only about 35 °C and did not exceed 75 °C.

The current policy of the Thai Royal



Dry season fire in savanna forest, Thailand. Note the arc effect of the flames (courtesy of P. Stott).

might otherwise invade and develop a more fire-resistant forest.

The drier areas of South-East Asia are occupied by deciduous dipterocarp savanna forests that often abut sharply into the neighbouring semi-evergreen forests, the abrupt divisions being maintained artificially by regular burning in the former. These dry forests, consisting largely of *Dipterocarpus* and *Shorea* species, are widely distributed between Burma and Vietnam. In Vietnam, the rural population clears the forest between January and March, before the wet season begins, using felling and burning techniques⁸.

Stott's¹ detailed studies of the effects of such fires in Thailand show that, as in the boreal forests of temperate regions, the major fuel is the dry litter on the ground. About 90 per cent of the leaves have been shed by the end of January and are a considerable fire hazard, especially when accompanied by dry grasses. Stott investigated the temperatures of the fires using 'thermacolor' paints⁹ which change colour at different temperatures. In most fires the highest temperatures attained were near the ground and reached 300–400 °C.

Forest Department is to prevent these dry-season fires because of the risks involved, especially in wildlife parks and sanctuaries. Stott regards this policy as inappropriate, because it permits the accumulation of litter and low-growing vegetation and so increases the likelihood of intense fires when they eventually take place. It would be better to learn from the American experience and institute a management regime in which regular and relatively frequent fires of low intensity are encouraged. □

1. Stott, P. J. *Biogeogr.* **13**, 345–358 (1986).
2. Oberle, M. *Science* **165**, 568–571 (1969).
3. Richter, D. D., Ralston, C. W. & Harms, W. R. *Science* **215**, 661–663 (1982).
4. Wright, H. E. *Science* **186**, 487–494 (1974).
5. Weaver, H. in *Fire and Ecosystems* (eds Kozlowski, T. T. & Ahlgren, C. E.) 279–319 (Academic, New York, 1974).
6. Cwynar, L. C. *Can. J. Bot.* **56**, 10–21 (1978).
7. Williamson, G. B. & Black, E. M. *Nature* **293**, 643–644 (1981).
8. Condominas, G. in *Human Ecology in Savanna Environments* (ed. Harris, D. R.) 209–251 (Academic, London, 1980).
9. Hobbs, R. J. & Gimingham, C. H. *J. Ecol.* **72**, 223–240 (1984).

Peter D. Moore is in the Department of Biology, King's College London (KQC), Campden Hill Road, London W8 7AH, UK.

Herpes infection and AIDS

SIR—In individuals infected with the human immunodeficiency virus (HIV), a prolonged period with no overt symptoms may be followed by the onset of AIDS (acquired immune deficiency syndrome) of which the virus is the cause. The reasons for this prolonged latent period are unclear but are of major importance for the understanding and control of AIDS. In this regard the recent report by Mosca *et al.*¹ that the promoter in the long terminal repeat (LTR) of HIV can be activated during infection with herpes simplex virus type 1 (HSV-1) is of obvious interest, especially as many AIDS patients exhibit opportunistic infections with HSV.

The fact, however, that the experiments were carried out using cell lines into which the HIV LTR (linked to a marker gene) had been stably introduced by transfection suggests that care must be taken in extrapolating these findings to cells latently infected with HIV itself. Thus, in similar experiments, a β -globin promoter introduced by transfection could be activated by subsequent HSV infection of the transfected cell line, although the endogenous β -globin gene was unaffected by such infection².

It seems therefore, that the chromatin structure of genes recently introduced by transfection may render them susceptible to a nonspecific induction by HSV infection, whereas the endogenous genes within the normal chromatin structure are unaffected. Although our laboratory has shown that a small number of endogenous cellular genes, with a normal chromatin structure, can be specifically activated during HSV infection^{3,4}, many more are nonspecifically activated.

The behaviour of an artificially introduced HIV promoter in response to HSV infection may not therefore be relevant to the regulation of the whole HIV genome in latently infected cells, particularly as transcription of other integrated viral genomes (for example those of polyoma and adenovirus) is known to be dramatically reduced when cells transformed with these viruses are infected with HSV⁵. Studies involving cells latently infected with HIV itself will be necessary to confirm the provocative suggestion of Mosca *et al.*¹ that infection with HSV could re-activate HIV and lead to the development of the symptoms associated with AIDS.

DAVID S. LATCHMAN

Department of Zoology and
Cell Biology,
University College London,
Gower Street, London WC1E 6BT,
UK

1. Mosca, J.D. *et al.* *Nature* **325**, 67–70 (1987).
2. Everett, R.D. *EMBO J.* **4**, 1973–1980 (1985).
3. Patel, R. *et al.* *Nucleic Acids Res.* **14**, 5629–5640 (1986).
4. Kemp, L.M. *et al.* *Nucleic Acids Res.* **14**, 9261–9270 (1986).
5. Stenberg, R.M. & Pizer, L.I. *J. Virol.* **42**, 474–487 (1982).

Claws' claws

SIR—Kitchener's¹ discussion of the possible habits of *Baryonyx*² is intriguing, but needs three comments.

First, *Baryonyx* might well have had difficulty in dealing with tough skins¹, but this seems unlikely to have been true of typical theropods. Modern crocodiles have this problem because their teeth are more or less conical, and not adapted for slicing; but those of normal theropods were bilaterally compressed 'steak-knife' teeth, with serrated anterior and posterior edges, like those of various thecodontians and the sebecosuchid crocodiles. In this case, a better modern model for functional comparison would be the Komodo monitor, *Varanus komodoensis*, which has similar, though acrodont, teeth.

Second, adaptation of the claws for breaking into carcasses¹ would only be meaningful in terms of unbreached examples: most carcasses available to a scavenger that was not a primary predator would probably have been those of animals killed by typical theropods, and already broken into by them. It is doubtful that there could have been enough selection pressure to produce this adaptation.

Third, regarding weight^{1,2}, the large size of grizzly bears does not prevent them from catching salmon, despite their being fast swimmers, and even trout, when not swimming quickly, can be taken by 'tickling' as they swim across a hand held open under water. Lateral nares placed well back² would also allow a crouching fisher to wait motionless with the tip of the snout¹ under water.

R.E.H. REID

Department of Geology,
The Queen's University of Belfast,
Belfast BT7 1NN, Northern Ireland

1. Kitchener, A. *Nature* **325**, 114 (1987).
2. Charig, A.J. & Milner, A.C. *Nature* **324**, 359–361 (1986).

Sequence similarity

SIR—Several recent articles have described members of a rapidly growing family of proteins, each of which contain a similar domain of roughly 200 amino acids that is likely to be involved in nucleotide binding. The majority are bacterial proteins known to be ATP-binding components of periplasmic transport systems^{1,2}, and this family has recently been found to include the product of the *mdr* gene, a membrane glycoprotein (the P glycoprotein) responsible for multiple drug resistance in a variety of mammalian cell lines^{3–5}. I would like to point out that the putative translation product of the gene *white* (*w*) in the fruit fly *Drosophila melanogaster*⁶ also fits very nicely into this group. The *white* locus is well studied genetically and molecularly because of the obvious and varied eye pigmentation phenotypes of its mutants, and is currently the focus of work on the mol-

ecular effects of transposable element insertions. Genetic evidence has suggested that the product of this locus functions in the deposition of pigment, but its precise biochemical role is unknown.

Homology between *w* and this family of proteins extends the unpublished observation of Gary Otto that *w* contains homology to a core of amino acids found in about a third of all proteins known to bind ATP. The homology involves amino acids 120–335 of *white* (five amino acids of the second exon and all but seven of the third⁶) and almost the entirety of bacterial proteins such as *hisP* and *malK*. The alignments of *w* with *hisP* and *malK* generated by the program DFASTP⁷ were assigned scores 13 standard deviations better than the mean score for proteins in the Protein Information Resource database. There is a comparable homology between *w* and other members of the family (*pstB*, *oppD*, *hlyB* or *mdr*). It is interesting that the region of homology is largely coincident with the third exon; perhaps the remainder of the *white* protein (the complete open reading frame contains 694 amino acids) carries out functions related to those of the other proteins of the homologous periplasmic transport systems.

This observation supports the notion that the *w* protein is involved in the active transport of pigments into pigment cells or pigment granules, an idea for which there is some experimental support⁸. There are two classes of pigment in the *Drosophila* eye, ommochromes (the brown pigments) and pteridines (the red pigments), and the question of how mutations in a single gene could eliminate the accumulation of both classes is longstanding. Here we see further similarity to the *mdr* locus, which confers resistance to a wide variety of compounds, and the histidine transport operon, which also functions in the transport of a variety of compounds, including histidine and basic amino acids. Although sequence homology does not provide a definitive biochemical explanation for the phenomenon of specific transport of multiple substrates, it does serve to make it less mysterious (see ref. 9 for a discussion of possibilities), and suggests that results obtained in any one of these transport systems should be of interest to those studying the other.

STEPHEN M. MOUNT

Department of Biological Sciences,
Columbia University, New York,
New York 10027, USA

1. Higgins, C.F. *et al.* *Nature* **323**, 448–450 (1986).
2. Doolittle, R.F. *et al.* *Nature* **322**, 451–453 (1986).
3. Gerlach, J.H. *et al.* *Nature* **324**, 485–489 (1986).
4. Gros, P., Croop, J. & Housman, D. *Cell* **47**, 371–380 (1986).
5. Chen, C.-J. *et al.* *Cell* **47**, 381–389 (1986).
6. O'Hare, K., Murphy, C., Levis, R. & Rubin, G.M. *J. molec. Biol.* **180**, 437–455 (1984).
7. Lipman, D.J. & Pearson, W.R. *Science* **227**, 1435–1441 (1985).
8. Sullivan, D.T. & Sullivan, M.C. *Biochem. Genet.* **13**, 603–613 (1975).
9. Ames, G.F.-L. *Cell* **47**, 323–324 (1986).

All sabretoothed carnivores aren't sharks

SIR—Jared Diamond¹ is unwise to combine evidence associated with *Homotherium's* probable prey and functional analysis of *Smilodon*, into a hit-and-run strategy of killing large prey for both, and implicitly for all, sabretooths. In this strategy, which is considered analogous to that of the great white shark, a large piece of flesh is torn from a vulnerable area of the surprised victim and the predator retreats to await the victim's death.

Smilodon's hit-and-run strategy, proposed by Akersten², involved the mandible in the operation of the sabres. After catching a fold of skin and flesh between the upper and lower canines, and with the mandibular flanges pressed against the body of the prey to provide stability, *Smilodon* drove its sabres through the fold. As the jaws closed the incisors and lower canines penetrated the base of the fold to free it from the body.

But *Homotherium* and *Smilodon* are distinctly adapted. Unlike *Smilodon*, all *Homotherium* teeth are serrated, suggesting an adaptation to shearing flesh³. The incisors lie in a rounded, interdigitating arc which is set forward of the canines to allow the taking of a bite from fleeing prey. *Smilodon's* lower canines and incisors form a nipping transverse pincer. *Homotherium's* dorsolaterally projecting lower canines are possibly adapted for slashing. Its upper canines are sharper, completely serrated, lower crowned and more laterally compressed.

Other sabretoothed carnivores show still wider morphological diversity. In the nimravids *Hoplophoneus* and *Eusmilus* the incisors are high crowned, conical and slightly recurved. As in *Homotherium*, all teeth are serrated and the upper and lower incisors form interdigitating arcs; the hypsodont sabres resemble those of *Smilodon* but are more laterally compressed. *Barbourofelis fricki* is as large as *Smilodon* with similarly hypsodont sabres but the morphology of its sabre is more strongly compressed laterally, with lingual and buccal grooves and serrated margins. The incisors and lower canines are serrated, compressed buccolingually and form arcs that interdigitate more completely than in *Homotherium*.

The sabretoothed marsupial *Thylacynmilus* possessed a sharpened, triangular-sectioned upper canine but only one large and one small lower incisor, or canine and incisor, and no more than two upper incisors⁴. Such 'incisors' were surely less able to penetrate the skin than those of any fissipede sabretooth, or even hyaenas. Diamond did not mention that *Hyaena*, *Crocota* and *Lycaon* bite segments of flesh from prey and let it bleed to death but do not possess sabrelike canines.

Diverse dental anatomies among sabre-

tooths suggest that, despite necessary functional similarities, precise adaptations varied. The hunting adaptations of these animals were surely diverse.

HAROLD N. BRYANT
C.S. CHURCHER

Department of Zoology,
University of Toronto,
Toronto, Ontario,
Canada M5S 1A1

1. Diamond, J.M. *Nature* **322**, 773–774 (1986).
2. Akersten, W.A. *Los Ang. County Mus. nat. Hist. Contr. Sci. no. 356* (1985).
3. Churcher, C.S. *Quaternaria* **8**, 263–275 (1966).
4. Churcher, C.S. *Aust. Mammal.* **8**, 201–220 (1985).

Waste disposal by plants

SIR—The provocative letter by Ford¹ notes that the abscission of leaves and leaf-like structures may function as a waste-disposal mechanism. As several times as much organic material is introduced into the soil by roots as by leaf and branch litter^{2,3}, roots provide another potential means of eliminating wastes.

The sloughing of root-cap cells is perhaps implausible for this function, because they continue to function normally by secreting lubricatory mucilage for up to three weeks after separation⁴, but root hairs have a lifetime of only a few days and the whole root cortex itself is usually shed later⁴. Five to ten per cent of the root mass may be released into the soil per day^{4,5}. Even the cortex cells die by autolysis rather than external compulsion⁴, although I have no evidence of whether their metabolism (and that of root hairs) changes before autolysis, as it does for leaves¹. Mycorrhizal death⁶ is also relevant², as 30–50 per cent of a plant's net primary production can go to the mycorrhizae⁶.

Waste disposal by roots is potentially more efficient than by leaves, because the excreted material necessarily remains within an individual plant's rhizosphere (extended to include the mycorrhizal network). The waste is then available for assimilation by suitable bacteria, and their consumption and metabolism by the soil microfauna provide a path for subsequent return to the plant⁷. There are obvious opportunities for coevolution by patch selection^{8,9} and specific mutualism.

LEIGH M. VAN VALEN

Biology Department (Whitman),
University of Chicago,
Chicago, Illinois 60637, USA

1. Ford, B.J. *Nature* **323**, 763 (1986).
2. Fogel, R. *Pl. Soil* **71**, 75–85 (1983).
3. Fogel, R. in *Ecological Interactions in Soil* (ed. Fitter, A.H.) 23–36 (Blackwell, Oxford, 1985).
4. Foster, R.C., Rovira, A.D. & Cock, T.W. *Ultrastructure of the Root-Soil Interface* (American Phytopathological Society, St Paul, Minnesota, 1983).
5. Rogers, W.S. *J. hort. Sci.* **43**, 527–528 (1968).
6. Harley, J.L. & Smith, S.E. *Mycorrhizal Symbiosis* (Academic, London, 1983).
7. Elliott, E.T., Coleman, D.C. & Cole, C.V. in *The Soil-Root Interface* (eds Harley, J.L. & Russell, R.S.) 221–229 (Academic, London, 1979).
8. Van Valen, L.M. *Evol. Theory* **4**, 231–233 (1980).
9. Wilson, D.S. *The Natural Selection of Populations and Communities* (Benjamin/Cummings, Menlo Park, 1980).

Body temperature and the thermodynamics of water

SIR—The suggestion by Paul¹ that the selection of mean body-temperature levels in homoiothermic animals is determined by the properties of water is a most interesting one. But his reasoning concerning the value of such an adaptation appears to be incomplete.

Paul says that because the specific heat of water is minimal at approximately 35 °C, an organism losing heat to the environment will have to do minimal work to restore its temperature to about this value. But for a given amount of heat lost by an organism, the same amount of heat will have to be generated to restore the loss, regardless of the temperature selected by the organism as its normal resting level. It is not the temperature change which is crucial to an organism but the heat lost or gained, as this heat represents energy which must be expended or dissipated by the organism in order to restore its initial level.

Furthermore, the cooling factor at around 35 °C will be greater than at any other temperature, as most of the cooling will take place as the result of water evaporation, either from the skin surface or by means of respiration. It is a case of roundabouts and swings: there is no apparent reason why this adaptation should have come about if the object is the maintenance of a given temperature level *per se*.

Although this seems to dispose of the argument that 35 °C is the optimum value for the maintenance of heat stability by the organism, it remains the optimum value for temperature lability. In other words it is easier to vary an animal's temperature at this value than at any other, because it will require least work. We might therefore seek for the reason for the selection of 35 °C in terms of the value to an organism of being able to alter its temperature easily.

There are a number of adaptive states that are either entered by animals varying their own temperatures, or are triggered by temperature changes from the environment. The induction of fever may be a powerful recovery mechanism in response to attack by hostile organisms. Hibernation is an adaptive state for some classes of mammal, and aestivation may also be beneficial. Shivering and sweating are adaptive responses to temperature change brought about from outside.

It remains to be shown, however, that these responses could not themselves have been adapted to take place at different temperatures. In other words, it is not immediately obvious that they determine the normal temperature of the organism rather than being determined by it. The explanation may lie in the fact that, because the creation and maintenance of

temperature differences is easiest at around 35 °C, it will be easier for an organism functioning at that temperature to emit or detect signals that are encoded by such differences. 35 °C is the most economical value in terms of energy consumption for the maintenance of good discrimination between temperatures for signal detection purposes. Signals at these levels will also be relatively free from neural noise.

R.J. BIRD

*School of Behavioural Science,
Newcastle upon Tyne Polytechnic,
Newcastle upon Tyne, NE1 8ST,
UK*

1. Paul, J. *Nature* **323**, 300 (1986).

SIR—I would like to challenge several of J. Paul's ideas in his proposal¹ that the body temperature of homoiotherms is close to 36°C because the specific heat of water is near a minimum at this temperature. First, not all homoiotherms have body temperatures near the 36°C of eutherian mammals. Monotremes average 31.7°C, while birds average 40°C (ref. 2). Furthermore, during aerobic exercise homoiotherms typically regulate their body temperature at 3–4°C higher than they do at rest³.

Second, Paul states that by regulating at 36°C an animal minimizes the cost of changing temperature because the specific heat of water is minimal at 35°C. According to his graph, however, this claim is based on a very small quantitative effect; as a first approximation, an animal regulating at 28°C will expend only 0.02 per cent more energy to raise its temperature

1°C than will an animal regulating at 36°C (this assumes the animal can store all the heat).

Third, Paul ignores the significance of temperature differences between the body and its environment for the energy budget. These seem to represent a more important quantitative effect. Again as a first approximation, the cost of maintaining body temperature above ambient temperature is proportional to the difference between the two. The costs for changing temperature are probably much less important than the magnitude of the animal–environment temperature gradient for an animal's energy economy.

While I doubt the exact role that Paul has suggested for the specific heat of water in determining the body temperatures of homoiotherms, the significance of the physical properties of water should be remembered as a potentially important factor in the evolution of body temperature. Interested readers may consult Calloway⁴, who has suggested that cells would function best at 40°C, Brock⁵, Hamilton⁶ and Heinrich³ for further points related to this subject.

R. D. STEVENSON

*Department of Zoology,
University of Cambridge,
Downing Street,
Cambridge CB2 3EJ, UK*

1. Paul, J. *Nature* **323**, 300 (1986).
2. Calder, W.A. *Size Function and Life History*, 431 (Harvard University Press, 1984).
3. Heinrich, B. *Am. Nat.* **111**, 623–640 (1977).
4. Calloway, N.A. *J. theor. Biol.* **57**, 331–344 (1976).
5. Brock, T.D. *Science* **158**, 1012–1019 (1967).
6. Hamilton, W.J. *III Life's Color Code*, 228 (McGraw-Hill, New York, 1973).

Tunable noise

SIR—Nittmann and Stanley¹ have introduced the concept of tunable noise to the dielectric breakdown model (DBM) growth process of Niemeyer *et al.*². Instead of adding to the growing structure at the first time a surface site is chosen, Nittmann and Stanley introduce the additional condition that growth will only take place at a surface site after it has been chosen s times, where $s = 1$ corresponds to the classical DBM algorithm. They observed that in their modified DBM algorithm, simulations with the condition $s > 1$ will grow thicker fingers as s increases and they call this phenomenon "tunable noise".

In the one-dimensional case, the DBM algorithm becomes identical to the Diffusion Limited Aggregation (DLA) algorithm of Witten and Sander³ as the number of sites becomes large. Recently, we provided a connection between DLA simulations and the fingering phenomenon of two-fluid displacement in porous media^{4,5}. In particular, we can identify the DLA algorithm with the displacement of a Newtonian fluid by an inviscid fluid (in the

absence of surface tension effects) in a model porous medium which has a single chamber with fluid capacity ϕ at each grid node where one discretizes the solution of the Laplace equation. To characterize the random nature of porous medium, the fluid capacities of the chambers are taken as independently and identically distributed random variables. The classical DLA algorithm corresponds to the case in which the probability density function for the fluid capacities ϕ is exponential,

$$f(\phi) = (1/\langle\phi\rangle) \exp(-\phi/\langle\phi\rangle)$$

whereas other densities $f(\phi)$ will correspond to porous media having different random structures. The case of a delta function distribution of fluid capacities ($\phi = \text{constant}$) is equivalent to a non-random medium in which fluid displacement is governed only by the solution of the Laplace equation — displacement in a perfectly uniform Hele-Shaw cell is a good realization of this limit.

Moreover, our unpublished data show that for a porous medium constrained to having a given mean fluid capacity $\langle\phi\rangle$, the exponential distribution is the most

random way in which the fluid capacity can be partitioned. In the language of Nittmann and Stanley, this exponential distribution (which corresponds to the classical DLA) has the highest possible noise level. The tunable noise condition of Nittmann and Stanley, parameterized by the value of s (> 1), corresponds to the case of having s chambers at each node, where the fluid capacity of each of the s chambers is still exponential. This decoration can be viewed as each node having a different statistical distribution of fluid capacities. The resulting fluid capacity per node is

$$\Phi = \sum_{i=1}^s \phi_i$$

and this random variable is readily shown to have the gamma density

$$F(\Phi) = \left(\frac{s}{\langle\Phi\rangle}\right)^s \frac{\Phi^{s-1}}{(s-1)!} \exp\left(-\frac{s\Phi}{\langle\Phi\rangle}\right)$$

with mean $\langle\Phi\rangle$. The case $s = 1$ reduces the exponential distribution as expected, whereas in the limit $s \rightarrow \infty$ the gamma distribution sharpens up towards the limit of a delta function. The concept of tunable noise in a DLA algorithm can be used to quantify porous media having different statistical properties.

DEREK Y. C. CHAN
BARRY D. HUGHES

*Department of Mathematics,
University of Melbourne,
Parkville, Victoria 3052,
Australia*

LINCOLN PATERSON

*CSIRO Division of Geomechanics,
PO Box 54, Mount Waverley,
Victoria 3149, Australia*

1. Nittmann, J. & Stanley, H.E. *Nature* **321**, 663–668 (1986).
2. Niemeyer, L., Pietronero, L. & Weismann, H.J. *Phys. Rev. Lett.* **53**, 1033–1036 (1984).
3. Witten, T.A. & Sander, L.M. *Phys. Rev. Lett.* **47**, 1400–1403 (1981).
4. Paterson, L. *Phys. Rev. Lett.* **62**, 1621–1624 (1984).
5. Chan, D.Y.C., Hughes, B.D. & Paterson, L. *Phys. Rev. A* **34**, 4070–4082 (1986).

Estimating guild size

SIR—Not only do we agree with Perry's modification¹ of our model of estimating guild size² but we had already incorporated the suggested modification into our simulation model, as reported at the British Ecological Society symposium in April 1986. The text of our contribution is in press³. As Perry suggests, the modification in the mechanics of the model produce slightly larger guild sizes which fit the field data more closely.

B. SHORROCKS
J. ROSEWELL

*Department of Pure and Applied Zoology,
University of Leeds,
Leeds LS2 9JT, UK*

1. Perry, J. *Nature* **325**, 202 (1987).
2. Shorrocks, B. & Rosewell, J. *J. anim. Ecol.* **55**, 527–541 (1986).
3. Shorrocks, B. & Rosewell, J. in *The Organisation of Communities Past and Present* (eds Gee, J. H. R. & Giller, P. S.) (Blackwells, Oxford, 1987).

Is lung surfactant protein a lectin – collagen hybrid?

SIR—Pulmonary surfactant lining the surface of alveoli is a complex mixture of lipids, carbohydrates and proteins. This lipoprotein complex is able to decrease surface tension at the air–liquid interface and thus helps maintain alveolar patency at low lung volumes (see ref. 1 for review). Recently the cDNA sequence of the major protein of canine and human pulmonary surfactant has been published; the deduced protein sequence was found to be rather unusual in that it predicts that the protein has a collagen-like region within its amino-terminal half and a non-collagenous globular domain within its

carboxy-terminal half^{2,3}. Although these features are reminiscent of the structures of collagen type IV and C1q^{4,5}, the globular domain of the surfactants seems to be unrelated to those of the other collagenous proteins.

In the gene of the human pulmonary surfactant-associated protein⁶ an individual exon codes for the carboxy-terminal globular domain, raising the possibility that exon-shuffling was responsible for the introduction of this exon into the gene of a collagen-like ancestor. This possibility prompted a comparison, reported here, with modules that have been used in the assembly of various vertebrate proteins^{7–9}.

Sequence comparison revealed that the

non-collagenous domain of pulmonary surfactant resembles a module that is present in hepatic lectins of various vertebrate species^{10–12}, the humoral lectin of the flesh-fly, *Sarcophaga peregrina*¹³, and rat cartilage proteoglycan⁹. The homology of the globular domain of canine and human pulmonary surfactant-associated protein with this module is underlined by the fact that of the 17 residues that are invariant among the various members of this module-family, 16 are conserved (identical or chemically similar) in both canine and human surfactant protein, as seen in the figure.

It should also be noted that in the alignment of the two pulmonary proteins the gaps coincide with the gaps dictated by the alignment of the various lectins and proteoglycan. In the region containing the four conserved cysteines (Cys²⁵–Cys¹²⁰) the homology with the various lectins and proteoglycans is 28–34 per cent. The homology of human and canine surfactant protein with animal lectins and proteoglycan is also supported by the high alignment scores (4.66–8.81 standard deviation units) determined with the ALIGN program¹⁴ (kindly carried out by Dr K.B.M. Reid, Oxford).

The similarity of the globular domain of pulmonary surfactant protein to a module common to various animal lectins raises the possibility that this protein may be involved in interactions with carbohydrate constituents of the lung surfactant complex.

LÁSZLÓ PATTHY

*Institute of Enzymology,
Biological Research Centre,
Hungarian Academy of Sciences,
Budapest, PO Box 7,
Hungary H-1502*

- Goerke, J. *Biochim. biophys. Acta* **344**, 241–261 (1974).
- Benson, B. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 6379–6383 (1985).
- Floros, J. *et al. J. Biol. Chem.* **261**, 9029–9033 (1986).
- Oberbaumer, I. *et al. Eur. J. Biochem.* **147**, 217–224 (1985).
- Reid, K.B.M. *Biochem. J.* **231**, 729–735 (1985).
- White, R.T. *et al. Nature* **317**, 361–363 (1985).
- Patthy, L. *Cell* **41**, 657–663 (1985).
- Südhoef, T.C. *et al. Science* **228**, 815–822 (1985).
- Doerge, K. *et al. J. Biol. Chem.* **261**, 8108–8111 (1985).
- Drickamer, K. *J. Biol. Chem.* **256**, 5827–5839 (1981).
- Drickamer, K. *et al. J. Biol. Chem.* **259**, 770–778 (1984).
- Spies, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 6465–6469 (1985).
- Takahashi, H. *et al. J. Biol. Chem.* **260**, 12228–12233 (1985).
- Dayhoff, M.O. *et al. Meth. Enzym.* **91**, 524–545 (1983).

cPSAP	VI SLHESILVVGRKVFSSNGDSINFDIQLCAGAGGQIAAPMSPEEN	60	YLGLVESPSGSD	70	FQYMDGAPV N
hPSAP	AI SLGGSIMTVGKVFSSNGDSITFDAIQEACARAGGRIAVPRNPEEN	60	YVGLTEGSPSGD	70	FQYMDGAPV N
rASGPR 1	RI CCPIVNWVEYEGSCYWFSSSVKVPWTEADKY	60	WIGLTDQNGP	70	WKWVDGTDTYETG
rASGPR 2	RI CCPIVNWVEYEGSCYWFSSSVKVPWTEADKY	60	WIGLTDQNGP	70	WKWVDGTDTYETG
hASGPR 1	RI CCPIVNWVEYEGSCYWFSSSVKVPWTEADKY	60	WIGLTDQNGP	70	WKWVDGTDTYETG
hASGPR 2	RI CCPIVNWVEYEGSCYWFSSSVKVPWTEADKY	60	WIGLTDQNGP	70	WKWVDGTDTYETG
PG	DEQCELGWTKFGHCYRHPDRE	60	WIGLTDQNGP	70	WKWVDGTDTYETG
chL	CGAQSQRQWEYFEGRCYFSLSRMSWHKAKAE	60	WIGLTDQNGP	70	WKWVDGTDTYETG
SpL	AAVVDLQKALDGRN	60	WIGLTDQNGP	70	WKWVDGTDTYETG
Consensus		W A C L			
cPSAP	EAVASIVKKNYNTYA	80	YLGLEVESPSGSD	90	FQYMDGAPV N
hPSAP	EATASIVKKNYNTYA	80	YVGLTEGSPSGD	90	FQYMDGAPV N
rASGPR 1	RFVVDQHMGLPNT	80	WIGLTDQNGP	90	WKWVDGTDTYETG
rASGPR 2	RFVVDQHMGLPNT	80	WIGLTDQNGP	90	WKWVDGTDTYETG
hASGPR 1	KFVVDQHMGLPNT	80	WIGLTDQNGP	90	WKWVDGTDTYETG
hASGPR 2	KFVVDQHMGLPNT	80	WIGLTDQNGP	90	WKWVDGTDTYETG
PG	EFVVDQHMGLPNT	80	WIGLTDQNGP	90	WKWVDGTDTYETG
chL	NFVVDQHMGLPNT	80	WIGLTDQNGP	90	WKWVDGTDTYETG
SpL	NAITDLVRRVVGKKSINL	80	WIGLTDQNGP	90	WKWVDGTDTYETG
Consensus		W G D			
cPSAP	YINWYGPGRGRGK	100	EQCVEYMTDG	110	QWNNKNCQYRLAICEF
hPSAP	YINWYGPGRGRGK	100	EQCVEYMTDG	110	QWNNKNCQYRLAICEF
rASGPR 1	FKWNR	100	EDCAHFTIDG	110	HWNDVFCRRPYRWVCEI
rASGPR 2	FKWNR	100	EDCAHFTIDG	110	HWNDVFCRRPYRWVCEI
hASGPR 1	FKWNR	100	EDCAHFTIDG	110	HWNDVFCRRPYRWVCEI
hASGPR 2	FKWNR	100	EDCAHFTIDG	110	HWNDVFCRRPYRWVCEI
PG	FEKWR	100	EDCAHFTIDG	110	HWNDVFCRRPYRWVCEI
chL	FEKWR	100	EDCAHFTIDG	110	HWNDVFCRRPYRWVCEI
SpL	FAYWSEN	100	EDCAHFTIDG	110	HWNDVFCRRPYRWVCEI
Consensus		F C		W N D C C	

Alignment of the sequences of canine and human pulmonary surfactant-associated proteins (cPSAP, hPSAP) with the sequences of rat and human hepatic asialoglycoprotein receptor (rASGPR, hASGPR), rat cartilage proteoglycan (PG), chicken hepatic lectin (chL) and the humoral lectin of *Sarcophaga peregrina* (SpL). The sequence of hPSAP shown in the alignment corresponds to the translated region of the fifth exon of the hPSAP gene. The residues shown in the last lines of the alignment are the consensus residues invariant in animal lectins and proteoglycan. The residues identical with those of cPSAP and hPSAP are boxed. The numbering of residues refers to the homology unit of asialoglycoprotein receptors and starts at the first tryptophan conserved in vertebrate lectins and proteoglycan. The sequences correspond to: amino acids 107–231 of the 231 residue mature cPSAP; amino acids 104–228 of the 228 residue mature hPSAP; segment 149–277 of the 283 residue rASGPR 1; segment 151–279 of the 291 residue hASGPR 1; amino acids 56–181 of the carboxyl-terminal 269 residues of PG; amino acids 78–203 of the 207 residues of chL and amino acids 22–159 of the 283 residue SpL.

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of less than 500 words and five references. □

Fitting the bill in biology

Paul H. Harvey *£50*

Ecology and Evolution of Darwin's Finches. By Peter R. Grant. *Princeton University Press*: 1986. Pp. 457. Hbk \$55, £36.70; pbk \$22.50, £15.10.

WHAT is the need for another book on Darwin's finches? Surely, as a museum curator once said to Peter Grant, "they have been done"? Their story is familiar to evolutionary biologists. Darwin visited the Galápagos Archipelago off the coast of Ecuador in 1835, and later suggested that the diversity of finch species he had seen there might be the result of an evolutionary radiation in which "one species had been taken and modified for different ends". Just over a hundred years later, David Lack's short stay on the islands resulted in a classic monograph, *Darwin's Finches* (Cambridge University Press, 1947), in which he classified 13 finch species on the Archipelago itself and another on the isolated Cocos Island (about 600 km north-east). Lack also speculated on the evolutionary origin of the group, which has remained one of the most cited instances of apparent allopatric speciation.

Lack was on the islands for less than four months and in the years after his visit he changed his mind about the causes of the main differences between the species, which are in beak sizes and shapes. In 1945, Lack believed that the differences between the species were not adaptive, but by 1947 he had decided that all the main beak differences "may be regarded as adaptations to differences in diet". Reviewing Lack's monograph in 1948, O. W. Richards argued that the solution to whether the beak differences were or were not adaptive would involve "studies of field populations, genetics, and variation. It is a problem that might be largely solved by teams of field workers dealing for a number of years with a particularly favourable example". Richards was correct, and in 1973 Peter Grant picked up the gauntlet and started working in the Galápagos.

Since then, with a talented group of collaborators, Grant has published about 50 papers reporting new findings about the population biology of Darwin's finches. Many of the papers would have been well cited even if their subject had not been Darwin's finches. For example, he

and his group have demonstrated powerful selective forces, some acting irregularly, that both change and maintain beak size distributions within populations. Together with field estimates of heritability, presented with appropriate caution, these findings demonstrate the likely causes of evolutionary change and stability in quantitative characters in different contemporary populations.

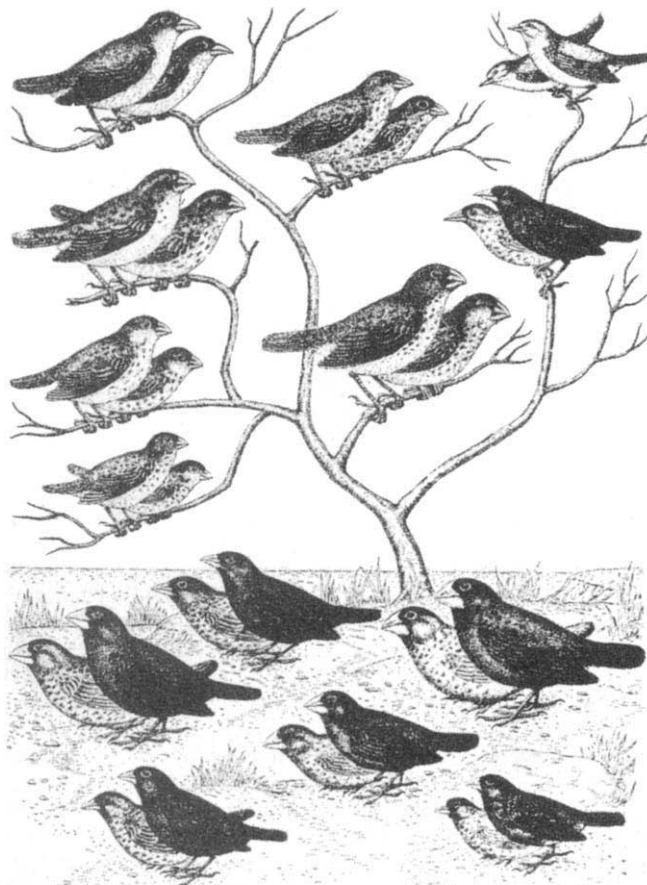
At first sight, the breadth of Grant's research seems puzzling. Surely, it is just a fascination with his study group that leads a research worker to publish papers which merely report the genetics of bill colour differences in young finches and the relative size of finch eggs? That conclusion would be incorrect. With a clear head for the problem to hand, Grant needed data on population structure which could best be obtained using genetic markers, and if

he was to describe evolutionary differences he needed to compare the ontogeny of development of the different species. Now he has brought his studies together in a clearly presented text that should have wide influence among evolutionary biologists.

Ecology and Evolution of Darwin's Finches reconstructs the radiation of the group using both historical records and contemporary data. One advantage of using the Galápagos as a study site is the relative absence of human disturbance. From Darwin's time to the present, no finch species has been lost and only a few island populations have become extinct (two are recorded). With such pristine conditions, Schluter and Grant were able to show how species are adapted to the particular islands they are found on. First, they demonstrated a relationship between beak size and diet (seed size). Second, they quantified the amounts of different sized seeds on various islands. Third, they showed that the particular species found on the islands were those that could best exploit the available food. Furthermore, the populations of the different species found on the various islands have become finely adjusted in beak size so as to match

the sizes of the seeds found there. Given their estimates of heritability for beak and body size measures, Price, Grant and Boag have been able to estimate the amount of selection that was necessary to produce both population and species differences. A spin off from this work has been the construction of a phylogenetic tree based on divergence through selection alone, which can be compared with those produced by Lack on morphological differences and by Yang and Patton on enzyme variation. The trees are almost identical.

For selection to result in genetic change, there must be genetic variation within populations. The usual sources of new genetic material are through mutation and migration. With Darwin's finches, Grant has documented rare but effective hybridization. In the absence of conspecifics, occasional immigrants to some islands mate with natives of different species, thus injecting new genetic variation into the local population. It seems that hybrids, though relatively uncommon, are viable. Why, then, is hybridization not more common, and how do species remain distinct? The answer seems to lie in the availability of food. On islands with populations of closely related species, Grant and



The 14 species of Darwin's finches, shown at about a quarter life size, which divide roughly into six ground species and eight tree species. The illustration is by Eric Mose and is reproduced with slight adaptation from David Lack's article on the finches in *Scientific American*, April 1953.

Ratcliffe have demonstrated mate choice for both song and morphology. In the past, when incipient species have come together on an island where the food resources are more effectively utilized by the pure than the hybrid populations (with their intermediate beak sizes), selection has favoured the evolution of ecological and reproductive character displacement.

Grant envisages populations of ancestral species being selected to differ in allopatry, the differences being dictated by food supplies. Subsequently, individuals may immigrate to an island, and find themselves occupying a different adaptive peak from native populations of their own species. Hybrids may find a paucity of food, possibly through being unable to compete effectively with pure-breeding birds from the two parental populations, and pre-mating isolation mechanisms are consequently favoured by selection. The result is speciation in sympatry resulting from adaptive divergence in allopatry.

The role of competition in speciation of Darwin's finches is still not altogether clear, but there is now ample evidence to demonstrate its importance in determining community structure, species distributions and morphological differences among populations of a species. Peter Grant will be remembered for challenging the evidence underlying the textbook cases of ecological character displacement, the finding that species occurring in sympatry are more dissimilar in some particular characters than when they occur apart. With careful attention to the data, his work presaged much of the subsequent controversy about the importance of interspecific competition as a determinant of pattern in nature. It is with the same critical approach that he has been able to turn the tables to reveal the likely importance of competition for understanding the population biology of Darwin's finches.

How, then, do Grant's conclusions differ from those of Lack? The main difference seems to be in their explanation for the causes of divergence in allopatry. Lack used Muller's theory of differential mutation to explain divergence. But Grant and his colleagues have been able to demonstrate that differences in beak morphology are closely tied to diet, which differs among islands as a result of variation in the availability of different food types.

Grant's synthesis provides a rich source of information and examples for contemporary population biologists. Having been the subject of important contributions during the nineteenth and twentieth centuries, Darwin's finches are now ready to take their part in the evolutionary biology of the twenty-first century. □

Paul H. Harvey is in the Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK.

Rules, regularities and reasoning

Stuart Sutherland

Induction: Processes of Inference, Learning, and Discovery. By John H. Holland, Keith J. Holyoak, Richard E. Nisbett and Paul R. Thagard. MIT Press: 1986. Pp.385. \$19.95, £19.95.

FROM Hume to Nelson Goodman, philosophers have been baffled by the problem of justifying induction. Professors Holland, Holyoak, Nisbett and Thagard neatly sidestep the issue by taking the pragmatic line that if it works it works. The reason we do not use the concept *grue* (green until time *t* and thereafter blue) is that it is no use to us. Their book is ambitious, for it seeks both to outline the mechanisms underlying induction and to show experimentally their effects on reasoning.

The authors believe that induction operates on a mental model of the world and consists of the formulation of production rules based on observed regularities, for example, "If it has wings, it flies". An existing rule is assumed to be correct until an exception occurs. The exception will be noted, or if it is accompanied by an unusual event that event will be used to form the basis for a new and more specific rule. The rules are hierarchical in that one can support another. Any rule activated at a given time is strengthened (or weakened) in proportion to the extent that it enables the organism to achieve its goals. The further back a rule is in the chain of active rules, the less it will be strengthened (shades of Clark Hull and secondary reinforcement).

It is unclear — at least to me — how they account for abduction (inferring an explanation for a set of phenomena), as practised by Albert Einstein and Hercule Poirot. They stress the role of analogy (and experimentally demonstrate its operation in everyday problem solving), but for every analogy that helps abduction there must be thousands that do not. In an attempt at precision, they present two computer programs instantiating some of their ideas, but the programs are described in such detail that they are hard to grasp. Moreover, they appear to operate with a very restricted range of possible events. One program apparently succeeded in becoming an expert in controlling the supply to a water system that sometimes leaked and whose optimal supply varied with the time of year, day of the week, and hour. Given how bad people are at controlling systems where factors interact, this may be no great achievement.

Induction is exceptionally and needlessly hard to understand: the order in which

ideas are presented often seems to be haphazard and the prose is cumbersome. When one strips away the fashionable but rebarbative terms such as default hierarchy (rules that admit exceptions) and *q-morphism* (a model of the world that is incomplete), the question arises how much is left that is new. The treatment of generalization seems to be just another way of saying that regularities are noticed. Moreover, the authors' insistence that rules change their strength gradually and in proportion to their usefulness raises problems, all of which they ignore. First, they cannot account for those moments of insight, in which one's way of looking at the world changes suddenly, dramatically and permanently. Second, it is not always obvious what constitutes a useful outcome, particularly given that curiosity or the desire to make sense of the world is one of man's primary goals and that preserving his self-esteem is another (and, indeed, one that may be responsible for many of man's less rational inductions). Third, like Hume, they can give no account of the concept of necessity which seems to play a role in much induction. But perhaps the biggest flaw is that they do not specify how the model of the world is represented and it is unclear what entities the production rules operate upon.

Despite these criticisms, there are some interesting points of detail in the theories. The authors point out, for example, that when confronted with several rules applicable to a situation, it is always the most specific rule that must be chosen for this will specify the exception, if there is one, to the more general rules. Again, they are aware that in all learning there must be constraints: there are always too many possible connections to remember them all. They have experimentally demonstrated a nice example of the use of one such constraint. Subjects who are told that a given member of the Barratos tribe is obese and brown are far more likely to conclude that all members of the tribe are brown than that they are all obese. The subjects already knew that members of a given race vary less in colour than in *avoir-du-pois*. Put more generally, in deciding whether a property belongs to all members of a class, people take into account their knowledge of its variability in that type of class.

The experimental sections of the book are, in general, clearer and more ingenious than the theoretical ones. They contain interesting studies on such topics as naive physics (most people believe that if someone is walking along and drops a coin, it will fall vertically to earth), on the difficulty people have in combining separate items of probabilistic evidence, and on inferring the causes of actions. There is a neat explanation for why out-groups are more stereotyped than in-groups: people have more experience of the variety with-

in their own group than of that within others. Unfortunately, however, despite much ingenuity, the theory is not rigorous enough to provide unequivocal explanations for many of the phenomena. As an example, consider the interesting finding that people are far more likely to attribute their own behaviour to an external event than they are that of other people, which they tend to attribute to internal dispositions. The authors argue that the situational factors that "drive and constrain one's own behaviour are far more salient than the same factors in others". But one could equally argue that a person's own mental states are far more salient than those of others, thus making the wrong predictions. It could even be that people tend to attribute their own behaviour to external events because they dislike blame

or, if they are modest, praise.

A further set of experiments examines how far people's ability to reason is affected by training in different subjects. Statistics and experimental design were found to be helpful, logic was not. It is reassuring to know that a good use has been found for statistics.

Almost any reader will find the experimental chapters of this book interesting, and the more determined will find flashes of insight in the theoretical chapters, but even the most dedicated may be hard put to piece the full theory together into a coherent whole. Maybe the authors should return to Hume, if only to study his perspicuous prose. □

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition, University of Sussex, Brighton BN1 9QG, UK.

Chinese features

M.M. Sweeting

Physical Geography of China. By Zhao Songqiao. Wiley: 1986. Pp.209 plus colour plates. £24.95, \$29.95.

Physical Geography of China is essentially an English-language version of Volume I of a 12-volume work in Chinese summing up the chief features of China's physical geographical environment. It is in many ways a team effort, embodying the work carried out by Chinese geographers since the founding of the People's Republic in 1949, but Zhao Songqiao with the help of the Science Press in Beijing and of Wiley has done a good job of organization and translation of the material. The text is clear and readable, and is divided into two parts. The first seven chapters deal with different aspects of the physical geography of the whole of China — climate, geomorphology, surface and ground water, soils and biogeography — while the second part of the book consists of regional studies of the country's natural divisions. This arrangement emphasizes the main factors which shape China's physical environment.

China occupies 6.5 per cent of the total land area of the world and has 23 per cent of the world's population. It is a vast country, containing a large variety of physical environments, and stretches from the Nansha coral islands near the Equator to the Heilong river (Amur) at 53°N, and from the islands of Taiwan and of the Pacific coast in the east to the Afghan frontier in the west. This immense area is divided into three natural realms — Eastern Monsoon China, the Nei Mongol–Xinjiang arid realm and the Qinghai–Xizang (Tibet) Alpine realm. These realms have been further subdivided into 7 natural divisions and 33 natural regions, and the

subdivisions form the basis for the second half of the book. Since 1978 a comprehensive land classification and mapping programme has been in progress, based on that used by CSIRO in Australia. The text is illustrated by samples of the land types covered by this mapping at a scale of 1:100,000; examples given include the dissected loess plateau area and the Turpan Basin.

The general chapters are particularly good on China's climate and water resources, the accompanying maps showing the various features of China's monsoon climate with its very cold winters and its extreme continentality. The section on geomorphology is a little less sharp but covers the vast area well and, like the whole book, is modern in its outlook; new ideas on peri-glaciation, glaciation and neo-tectonics are well represented. The chapter on the classification of the soils is based on the FAO–UNESCO System.

In the second part, each of the 33 natural regions with its subregions is identified and described in terms of its physical fea-

tures and areal differentiation. In addition, the regions are assessed for their potential for agricultural development and settlement. The descriptions are well balanced and introduce the main characteristics of each region — climate, geomorphology, vegetation and potential biological resources. The results of the work of the Desert Research Institute at Lanzhou and of the Symposium on Qinghai–Xizang in 1980 are incorporated into the excellent sections on north-west and west China. Considerable attention is also given to the developing areas of the cool temperate north-east and tropical south China.

Almost all of the many references given at the end of each section are in Chinese, indicating how much work is now available to those geographers who can read the language. The numerous maps, profiles and tables are very clear, and there are 15 pages of well-chosen colour photographs which depict aspects of the physical geography and vegetation. Eleven Landsat images are included, illustrating some striking areas such as Lop Nor. There is, too, a useful topographic map of China, though not all of the place names mentioned in the text are included on it. Modern pinyin terminology is used throughout (rather than the older Wade–Giles romanization), and it is helpful for English readers to be introduced to the administrative units in Chinese and to the more commonly used Chinese words and units of measurement. A glossary of place names in Chinese is also included.

The presentation of *Physical Geography of China* may be orthodox; but this is the best modern book on the subject and demonstrates clearly the great intellectual developments made by Chinese geographers in the past 30 years. □

M.M. Sweeting is Reader in the School of Geography, University of Oxford, Mansfield Road, Oxford OX1 3TB, UK, and a Fellow of St Hugh's College, Oxford.



Above it all — Landsat image of the area around Dongting Lake, south-central China.

The meeting with Dr Cameron

R. J. Tayler

Cosmogonical Processes. Edited by W. D. Arnett, C. J. Hansen, J. W. Truran and S. Tsuruta. VNU Science Press: 1986. Pp. 300. DM 135.

A. G. W. (Al) Cameron has made many notable contributions to astrophysics in the past 30 years. He was trained as a nuclear physicist, but a latent interest in astrophysics was activated by Paul Merrill's 1952 discovery of the unstable element technetium in stars. Cameron started to work on nuclear astrophysics and in 1957, at about the same time as Burbidge, Burbidge, Fowler and Hoyle published their celebrated paper on stellar nucleosynthesis in *Reviews of Modern Physics*, he wrote a Chalk River report which identified the crucial processes in the formation of the elements. He also stressed the probable importance of neutron stars and, with students and colleagues, calculated their properties before the discovery of pulsars caused most astronomers to believe in their existence.

In March 1985 four of Cameron's former students organized a symposium at Boulder, Colorado, to celebrate his sixtieth birthday, and this volume contains written versions of all but one of the talks together with an autobiographical sketch by Cameron himself. The book contains review articles on a variety of topics of current astronomical interest, most of them being ones to which Cameron has made significant contributions. The articles are generally written in a manner which makes them accessible to readers who are not experts in the fields concerned, and the book is one of the better examples of published conference proceedings.

One recurring theme concerns the questions of what the Universe is made of and how much of it there is. There is a theoretical prejudice that the Universe should have a critical density such that it will just expand for ever. In this case, much of the matter is probably composed of non-baryonic elementary particles of a type whose existence has not yet been confirmed. Press describes how such particles might accumulate in the centre of the Sun, and might then provide a solution to the long-lasting solar neutrino problem. Rees discusses how galaxy formation might have been affected by this non-luminous matter, and Fowler asks whether the age of the Universe can be compatible with the ages of objects in it if the Universe does have critical density.

The origin of the chemical elements,

Cameron's early interest, is the subject of several contributions. Pagel reviews the chemical evolution of galaxies and explains how the observed variation of chemical composition with position and with stellar age may be explained by infall of unprocessed gas, by outflow of processed material or by a variation with time of the stellar mass function (or, more likely, by a combination of all three). Clayton discusses the peculiar isotopic abundances which are found in some meteorites and Hillebrandt's contribution on nucleosynthesis in supernovae indicates that, while substantial progress has been made since 1957, much remains to be understood.

The remaining chapters cover other topics in which Cameron has had a particular interest: supernovae and neutron

stars and the origin of cosmic rays, and the formation of stars and of the Solar System. Modestly, the four organizers of the meeting chose not to present any papers or to write any articles for the book. However, anyone familiar with the field will know that they have all made major contributions to the subject and that Cameron's contribution to astronomy through the training and inspiration of research students has been as important as his own distinguished research. This is the clear message of the book, which is alike a worthy tribute to Cameron and a very useful summary of the present state of research in the areas discussed. □

R. J. Tayler is a Professor in the Astronomy Centre, University of Sussex, Brighton BN1 9QH, UK.

Advice in analysis

Rodger Staden

Software Directory for Molecular Biologists: A Complete Guide to the Selection of Computer Software for the Management and Analysis of Molecular Sequences. By Christopher J. Rawlings. Macmillan, London/Stockton, New York: 1986. Pp. 412. £40, \$80.

It is ten years since the publication of the articles that described the rapid and simple methods used to determine sequences of DNA. So efficient are these techniques that it has been necessary to create international data libraries to record the results. These collections have grown so that the European library now contains over eight million nucleotides, and the record for the longest individual sequence has tripled every two or three years.

Computers are used to help determine and analyse all this genetic data, but whereas the sequencing techniques were picked up fairly quickly, the spread of computer programs for handling the data was hampered by several problems. The users of the software were computer-naïve, and lacked suitable hardware; programs were not easily run on different machines, and it was hard to find people to convert them for local facilities. By modern standards the early programs were not user-friendly, and the concurrent emergence of personal computers showed what could be done. These factors, combined with the decision of *Nucleic Acids Research*, the journal that publishes most of the work in this area, not to referee formally papers submitted for its special computer issues, have led to a proliferation of software for analysing sequences. So we have an area where computers are essential, where users are computer-naïve and the software is abundant but of

unknown usefulness, and thus an obvious need for a source of information on the subject. This book aims to fill the vacant niche.

Its nomenclature and glossary cover the field, and explain the relevant computer terminology including that used in the software employed for sequence analysis; it outlines the use of word-processing packages, data-management systems, terminal emulation and even spreadsheets. More than 75 per cent of the contents is taken up by the well-cross-referenced directory of software, with 60 per cent of the references being from the special computer issues of *Nucleic Acids Research*.

For those knowing the field, the book will be very helpful as a single source of reference. But for its intended audience of newcomers wishing to choose software and hardware, although useful, it is weak on two important points. First, it does not address the problem of unreferenced publications, as no real opinions are given about the value of the packages described. Secondly, this is a fast-moving area, and a book — which is inevitably already out of date, and in which I could find no mention of future updates — is possibly an inappropriate vehicle for much of the information provided. □

Rodger Staden is at the Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.

Computing applied

Other recent books on the use of computers in science:

- *Chemistry by Computer: An Overview of the Applications of Computers in Chemistry* by Stephen Wilson (Plenum; \$37.50).
- *Computer Software Applications in Chemistry* by Peter C. Jurs (Wiley; \$34.95, £33.75).
- A second edition of *Microcomputers and Physiological Simulation* by James E. Randall (Raven; \$34).
- *Scientific and Engineering Applications with Personal Computers* by Raymond Annino and Richard Driver (Wiley; \$47.50, £45.65).

Revised history of early Tertiary plate motion in the south-west Pacific

Joann Stock & Peter Molnar

Department of Earth, Atmospheric, and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 USA

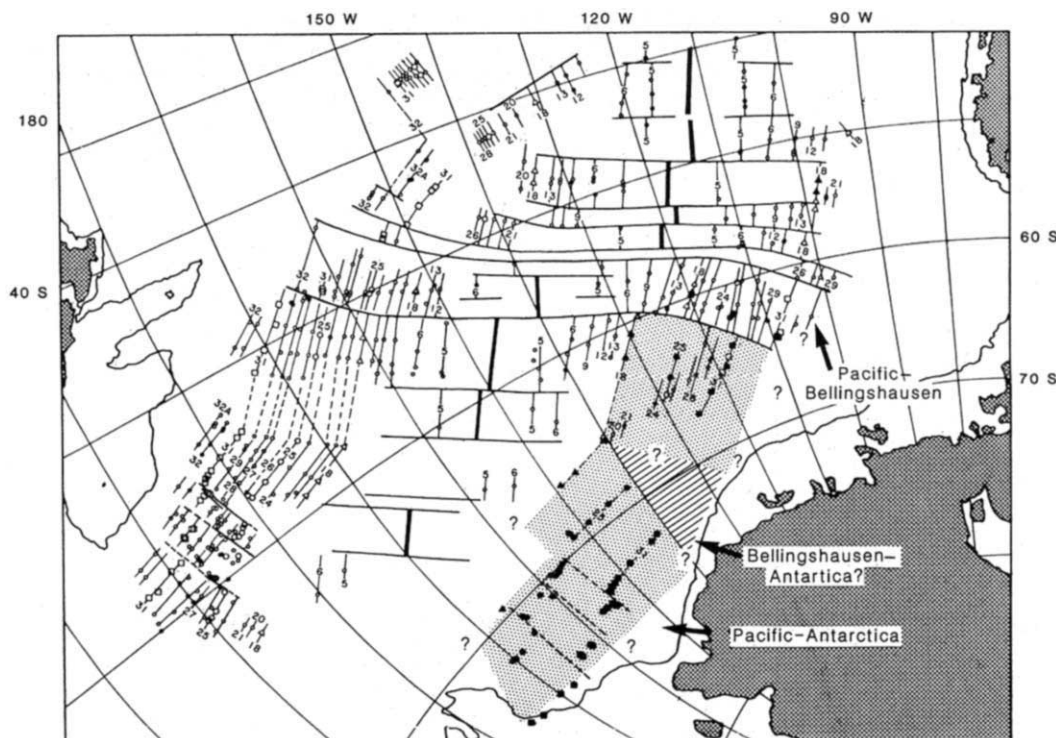
Reanalysis of early Tertiary magnetic anomalies on the Pacific plate south of the Campbell Plateau indicates that in early Tertiary time there was a previously unrecognized triple junction of the Pacific, the Antarctic, and a third plate now beneath the Bellingshausen sea. Corresponding revised parameters for Pacific–Antarctica motion do not require early Tertiary displacements within Antarctica or New Zealand, but suggest a change in direction of the Pacific plate over the hotspots of approximately the same sense and timing as the Hawaiian–Emperor bend.

ATTEMPTS to reconstruct the major lithospheric plates for late Cretaceous through Eocene time have been plagued by the demonstrable existence of a missing plate boundary, of unknown location and displacement, between the North Pacific and East Antarctica. Partly from geological arguments^{1,2} this boundary was first thought to lie between East and West Antarctica^{3,4}. Discrepancies in palaeo-spin axes, inferred both from palaeomagnetism and from sediment facies on the Pacific plate, seemed to imply at least 1,000 km of relative motion at such a boundary^{5,6}, but recent palaeomagnetic results in West Antarctica, when compared with those of East Antarctica, prohibit such large displacements⁷. The suggestion that this boundary lay along the Eltanin fracture zone system^{5,6} was ruled out by the distribution of magnetic anomalies east and west of it⁸, and the possibility that it lay north of the Eltanin system has been ruled out by the spacings of magnetic anomalies and the orientations of fracture zones in the north and south Pacific^{9,10}. On the basis of the spreading history south of the Campbell Plateau, we suggest that the boundary lay north of Marie Byrd Land,

off the West Antarctica coast, through the area now occupied by the Amundsen and Bellingshausen Seas.

There is a subtle bight in the trends of magnetic anomalies 18 to 32 on the Pacific plate southeast of the Campbell Plateau (Fig. 1). Molnar *et al.*⁴ rejected the suggestion that this bight reflects the existence of two separate plate boundaries and inferred that oblique spreading had occurred along at least one segment of the Pacific–Antarctic ridge. Here, we develop more fully the idea that, in fact, the Campbell Plateau magnetic bight does result from the spreading of the Pacific plate from two separate plates: the Antarctica plate, which includes both East and West Antarctica, and a third, previously unrecognized plate (the Bellingshausen plate), whose remnants in the Bellingshausen and Amundsen seas are now attached to the Antarctic plate (Fig. 1). First, steady-state oblique spreading is generally confined to ridges with half-spreading rates of 15 mm per yr or less, with a geometry such that the net length of ridges and transform faults is reduced¹¹; half-spreading rates for the Pacific–Antarctica ridge in this area averaged 36 mm per yr

Fig. 1 Identifications of Tertiary magnetic anomalies in the South Pacific, after Molnar *et al.*⁴. Open symbols are unrotated. The Campbell Plateau magnetic bight is located at the bend in trend of anomalies 32 to 18 south-east of New Zealand. Solid symbols are Pacific plate anomalies 31 and 25 rotated to Antarctica by Pacific–Bellingshausen rotation parameters, and anomaly 18 rotated by Pacific–Antarctica rotation parameters¹⁴, to show the predicted position of the corresponding anomalies on Antarctica. Light shading indicates parts of the present sea floor of the Antarctica plate, inferred to have been formed by Pacific–Antarctica and Pacific–Bellingshausen spreading. Medium shading shows where seafloor might have been formed by Antarctica–Bellingshausen spreading, if this triple junction had been of the ridge–ridge–ridge type. Predicted motion of the Bellingshausen plate relative to Antarctica would have been about 23 mm per yr at the triple junction, in a direction about N40°E, during this interval. Map is an oblique Mercator projection about 70.0° N, 72.0° W, the pole of rotation for Pacific to Antarctica at anomaly 5 time¹⁴.



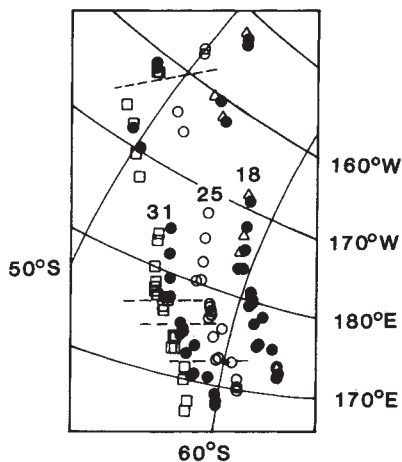


Fig. 2 Crossings of anomaly 18 (triangles), anomaly 25 (circles) and anomaly 31 (squares) on the Pacific plate, on both sides of the Campbell Plateau magnetic high. Fracture zones are shown by thin broken lines. Solid circles are anomaly 25 points rotated by the 'stage pole' parameters derived from reconstructions of anomalies 18, 25, and 31 based on points from both sides of the ridge north-east of the high¹⁴, which we now attribute to Pacific-Bellingshausen spreading. Note that the stage pole for anomaly 25 to anomaly 31 fits points north-east of the high but misfits those south-west of it. Map is an oblique Mercator projection about 47.87° S, 107.21° E, the stage pole derived here for Pacific-Antarctica motion.

between the times of anomalies 25 and 31, and the inferred obliquity would be of the opposite sense. More importantly, magnetic anomalies 25 and 31 on the Pacific plate cannot be reconstructed to one another without unacceptably large mismatches along segments north or south of the high.

Previous reconstructions^{4,12-14} of early Tertiary magnetic anomalies (older than anomaly 18) between the Pacific and Antarctica plates were based primarily on magnetic anomalies and fracture zones north-east of the Campbell Plateau magnetic high. The equivalent magnetic anomalies have not been recognized along the sparse ship tracks on the Antarctic side south-west of the high. The anomalies on the Pacific plate south-west of the high were used in earlier studies primarily as a constraint

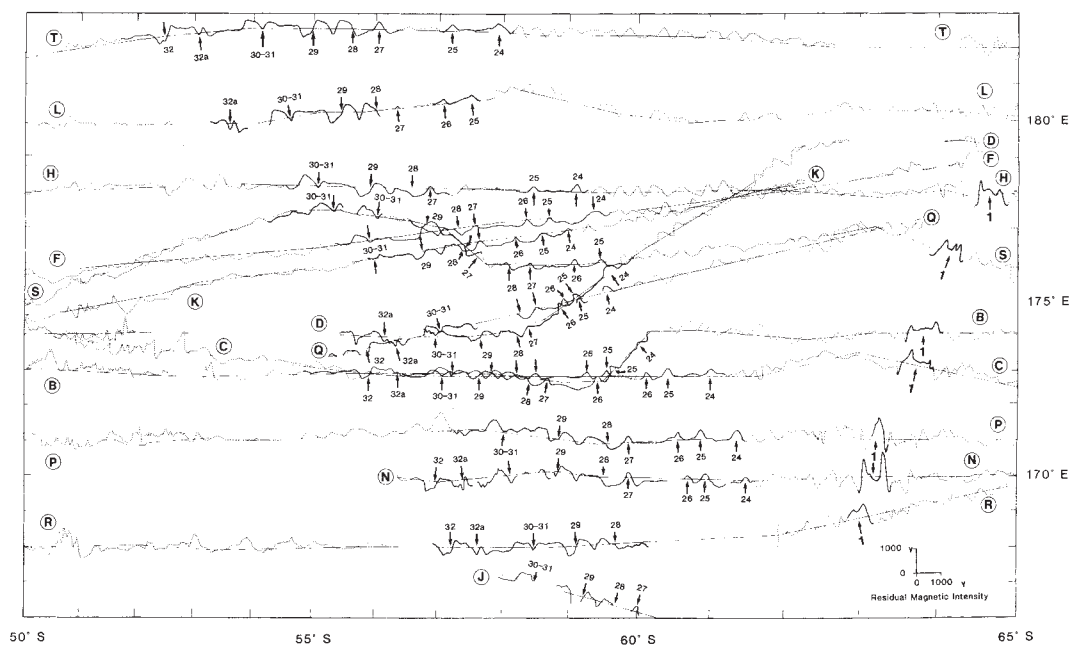
in maintaining symmetric spreading. When these previous reconstructions¹⁴ are used to calculate a 'stage pole' and 'half angle' describing the relative motion between the times of these anomalies, the resulting rotation of anomaly 25 to 31 on the Pacific plate misfits by up to 150 km south-west of the high (Fig. 2). A comparable mismatch of fracture zone trends also results. The differences in the separations of anomalies 31 and 25 and in the orientations of fracture zones north-east and south-west of the high would be produced by two plates only if there had been asymmetric, oblique spreading with propagating ridges and transform faults of just the right sense and rate to produce apparent fracture zones perpendicular to the magnetic anomalies south-west of the high. This seems very unlikely, and we conclude that there must have been three plates in this area between at least the times of anomalies 31 and 25. The early Tertiary magnetic record north-east of the high, and rotation parameters derived for it, thus represent motion between the Pacific and Bellingshausen rather than the Pacific and Antarctica plates.

Pacific-Antarctica motion

We reexamined the available magnetic anomaly profiles from this part of the Pacific plate in order to determine how such a revised history might be constrained. Original profiles^{4,12,15-18} of magnetic anomalies from cruises in this area were reexamined for anomalies 18 through 32, and fracture zone crossings were picked from bathymetric expression or from missing or repeated magnetic anomalies. An uncertainty was assigned to the position of each crossing according to the quality both of the expression on magnetic or bathymetric profiles and of the navigation. Anomalies 32 through 24 are generally well recorded on these profiles, but, because many of the tracks predate satellite navigation, their locations may be uncertain by up to 40 km, as can be seen from the correlations between adjacent and/or crossing tracks (Fig. 3). In cases of evident discrepancy, we relied more heavily on the better navigated tracks.

These anomalies were used to compute independent 'stage poles' and 'half angles' for Pacific-Antarctica relative motion. We fit crossings of anomalies 18 (42.01 Myr on the DNAG timescale^{19,20} and 25 (58.94 Myr) to the reversed polarity interval between anomalies 30 and 31 (68.47 Myr). These anomalies are clear, and because we have studied them in other oceans, we can combine reconstructions of them directly with those in other oceans without introducing additional uncertainties by interpolation.

Fig. 3 Mercator projection showing residual magnetic intensity plotted perpendicular to ship track for the *Endeavor* cruises reexamined in this study. Lines that are published elsewhere perpendicular to ship track (refs 4, 12, 16-18) were also reevaluated but are not included on this plot. Numbered arrows indicate the positions of magnetic anomalies used in this study; '1' is the central anomaly. Magnetic intensity values are shown by a dotted line where anomalies are not recognizable. Hatched zones are approximate positions of fracture zones. Much of the disagreement between crossing or adjacent traces may be due to navigation errors.



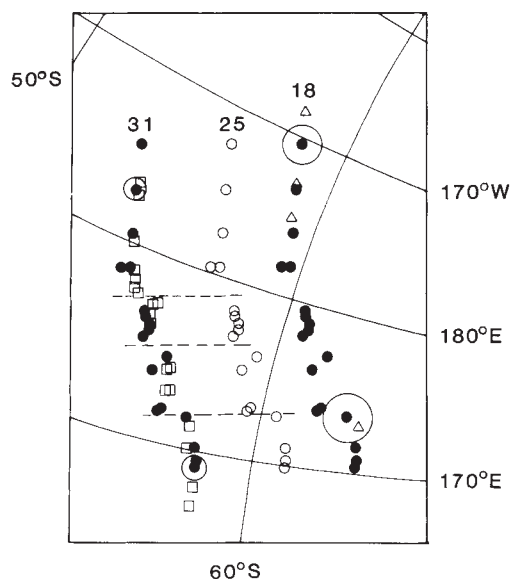


Fig. 4 Crossings of anomalies 18 (triangles), 25 (circles) and 31 (squares) on the Pacific plate south-west of the Campbell Plateau magnetic bight. Thin broken lines are approximate positions of fracture zones. Solid circles are anomaly 25 points rotated about 47.87° S, 107.21° E (the stage pole derived here for Pacific–Antarctica spreading) by -3.29° to anomaly 18 and by 4.21° to anomaly 31. Ellipses around rotated points indicate the sizes of the 95% confidence regions: 40 km radius for the fit of anomalies 25 and 31, and 80 km radius for the fit of anomalies 18 and 25. Map projection is the same as in Fig. 2.

Anomaly 25 was fit to anomaly 31 initially using Hellinger's¹³ method and subsequently by adding small rotations to produce what we considered to be our best fit (Fig. 4). This was necessary because uncertainties in navigation and sparse fracture zone crossings prevented the best fit from being well constrained by Hellinger's method. Nevertheless, the fits yielded by his routine lie well within the uncertainties that we assign to our best fits. The reconstruction of anomaly 18 to anomaly 25 is not as reliable as that for anomalies 25 and 31 because there are very few crossings of anomaly 18 south-west of the bight, and no clear crossings of fracture zones of the appropriate age. To reconstruct anomaly 18 to anomaly 25, we have used the pole position that we derived for anomaly 25 to anomaly 31, with the angle adjusted to bring crossings of anomaly 18 to those of anomaly 25 (Fig. 4). This is consistent with the parallelism of the easternmost segment of the reconstructed plate boundary from anomaly 32 to anomaly 18 time (Fig. 1). The resulting reconstructions of anomaly 18 to anomaly 25 and of anomaly 25 to anomaly 31 differ from those derived from previous reconstructions of the entire ridge¹⁴ and from reconstructions of anomaly 18 to anomaly 25 calculated using only the Pacific plate anomalies north-east of the bight⁹.

Cessation of Antarctica–Bellingshausen motion

Use of these 'stage poles' in global reconstructions requires knowledge of the exact time when the plate configuration changed and Bellingshausen–Antarctica relative motion ceased. Anomalies between 18 and 6 are not well recorded in this area, but other evidence suggests that this transition began at approximately the time of anomaly 18. First, the apparent parallelism of the anomaly trends south-west of the bight, from anomaly 31 to anomaly 18, suggests that these were all formed in a similarly oriented spreading system, with no hint of the major reorientation required to rotate the ridge crest into its configuration at the time of anomaly 6. The dramatic increase in roughness of the topography of the seafloor younger than anomaly 18 on this part of the Pacific plate⁴, and the change from sinistral

to dextral offset along fracture zones¹⁵ at about the time of anomaly 18, also indicate that a substantial decrease in spreading rate accompanied by a change in direction was occurring at this time. Moreover, the spacings between the few identifications of anomalies 5, 6, and 18 lying south and west of the bight along the Pacific–Antarctic ridge are predicted accurately by rotation parameters determined from magnetic anomalies and fracture zones north and east of the bight. Fracture zones seen on bathymetric and Seasat²¹ compilations of this area appear continuous from the ridge crest to approximately the position of anomaly 18. We assume in this paper that Bellingshausen–Antarctica relative motion ceased at anomaly 18 time, but we recognize that the transition was probably not instantaneous, and may have begun as early as anomaly 21 time, when a major plate reorganization took place in the south-east Pacific^{8,12}.

Reconstructions of the positions of Antarctica relative to the Pacific plate at the time of anomalies 25 and 31 (Fig. 4) were obtained in two steps: first, by rotating Antarctica to the Pacific plate at the times of anomaly 18 and second, by rotating it about the stage pole derived here for anomaly 18 to anomaly 25 or anomaly 31 on the Pacific plate. Rotation angles are twice those needed to rotate the respective anomalies on the Pacific plate into correspondence; thus we assume symmetric spreading. The final reconstructions of Antarctica to the Pacific plate contain uncertainties due to (1) the anomaly 18 reconstruction for Pacific to Antarctica; (2) the reconstruction of anomaly 18 to anomalies 25 or 31 on the Pacific plate; (3) the assumption that Bellingshausen–Antarctica motion ceased at anomaly 18 time; and (4) the assumption that all of these plates have been essentially rigid since the late Cretaceous. We have quantified the uncertainties in the first two of these assumptions²² using a method^{23,24} that incorporates the geographical distribution and the uncertainties in the individual data points.

Circumstantial evidence suggests that the third assumption is correct. We tried to test it definitively using the Pacific–Australia spreading lineations present in the Emerald basin¹² (south-west of the South Island of New Zealand), but the observed spacing and orientation of anomalies 13 and 18 are within the uncertainties of predicted Australia–Antarctica–Pacific plate motion, regardless of whether Antarctica–Bellingshausen motion ceased at the time of anomaly 18, at the time of anomaly 13, or in between. Global reconstructions show that this change in Pacific–Antarctica spreading direction implies a change in direction of motion of the Pacific plate over the hotspots, of the same general sense and close to the time²⁵ of the bend in the Hawaiian–Emperor seamount chain (43.1 ± 1.4 Myr, ref. 26). This correlation suggests that Antarctica–Bellingshausen relative motion ceased at about the time of anomaly 18 (42 Myr).

The assumption that no other plate boundaries existed in or near Antarctica is also difficult to test, and two results of the reconstructions suggest that there may have been a diffuse Tertiary boundary there, albeit with only a small amount of displacement. First, the revised fit of the Campbell Plateau to West Antarctica for the time of anomaly 31 (68.47 Myr) contains an overlap of the inferred position of the ridge crest at this time with the 2-km isobath in the Ross Sea. (If Antarctica–Bellingshausen motion continued beyond the time of anomaly 18, this overlap would be even larger.) Unless this shallow sea floor is underlain by thick oceanic crust, like that beneath Iceland or some aseismic ridges and plateaus, this overlap indicates a problem either with this reconstruction or with the assumption that the current shape of the Antarctic margin was inherited from late Cretaceous rifting. Cenozoic deformation and volcanism occurred in the Ross Sea area^{27,28} and in Marie Byrd Land²⁹; the amount and timing of associated extension is not yet well constrained, but may have caused some distortion of the Antarctic continental margin. The existence of a minor plate boundary in Antarctica is also suggested by a small discrepancy between the calculated and observed tectonic history of New Zealand, discussed below.

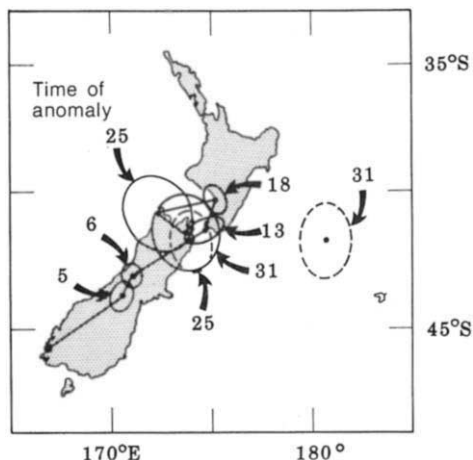


Fig. 5 Past positions of a point on the Pacific plate (south of the Alpine fault) relative to the Lord Howe Rise (North of the Alpine fault) at the times of anomalies 5, 6, 13, 18, 25, and 31. Ellipses are 95% confidence limits for these past positions. Complete parameters for the Pacific–Antarctica–Australia plate circuit, used to derive these reconstructions, are published elsewhere²². We assume no motion within Antarctica in the Tertiary period. Solid lines connect positions calculated from the newly derived Pacific–Antarctica rotation parameters (Fig. 4; see text for discussion). Positions surrounded by dashed uncertainty ovals are based on previous parameters¹⁴ for the times of anomalies 25 and 31. Note that the revised parameters indicate very little motion across this boundary in the early Tertiary, with none required before anomaly 25 time. By contrast, the large amount of motion implied by previous reconstructions exceeds that consistent with the geological record there.

Early Tertiary plate boundaries

Three plate boundaries are currently active in the south-west Pacific: the Pacific–Antarctic ridge between the Antarctica and Pacific plates, the south-east Indian ridge between Australia and Antarctica, and the Australia–Pacific plate boundary through New Zealand. In the scenario we present, these were the only active boundaries from anomaly 18 time to the present.

The differing evolutions of the south-east Indian Ridge and the Pacific–Antarctica boundary for the period between the times of anomalies 31 and 25 require a third plate boundary to have been active in the south-west Pacific. This was noted by previous authors using the Pacific–Antarctica spreading parameters that are here assigned to Pacific–Bellingshausen spreading^{4,12,14}, but it is also required by our revised Pacific–Antarctica reconstructions. Some motion must have occurred between the Pacific and Lord Howe plates prior to the time of anomaly 18, in order to form at least 50 km of older sea floor, measured perpendicular to spreading direction, in the Emerald Basin¹². This third plate boundary, which would have experienced at most a few hundred kilometres of displacement during this period, probably continued through New Zealand, manifested by subsidence and faulting beginning in middle Eocene time³⁰ (50 Myr).

Previous reconstructions of the south-east Indian and Pacific–Antarctic boundaries required another boundary in this area during the opening of the Tasman Sea (anomalies 33 to 24; 80 Myr to 55 Myr). Because deformation in New Zealand did not start until middle Eocene³⁰ or late Eocene–early Oligocene^{31–33} time, most previous workers placed this boundary between East and West Antarctica, through the Ross Sea–Weddell Sea region (for example, refs 4, 5, 14, 34; see ref. 12 for an exception). This trans-Antarctica boundary was thought to have been active until about the time of inception of the Pacific–Antarctica boundary through New Zealand. At least 150 km of motion was required across the postulated East Antarctica–West Antarctica boundary, and ‘best-fit’ reconstruc-

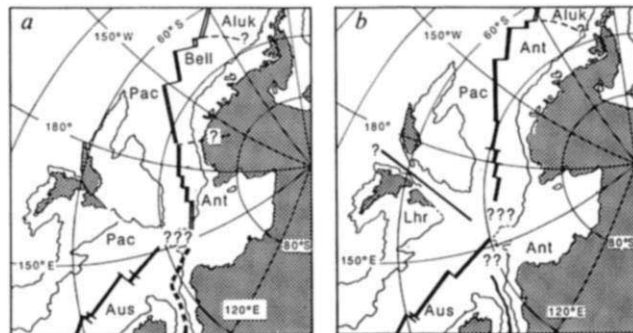


Fig. 6 Reconstruction of plate boundaries in the south-west Pacific for the time of anomaly 31 (68.47 Myr). *a* Revised reconstruction of the Pacific plate (Pac), the Bellingshausen plate (Bell), and the Aluk plate (Aluk) to the Antarctic plate (Ant) based on the revised Pacific–Antarctica spreading history and data west of the Campbell Plateau magnetic bight. *b* Reconstruction based on previously published Pacific–Antarctica spreading parameters derived from the data east and north of the Campbell Plateau magnetic bight¹⁴. The spreading centre in the Tasman Sea, and a slow rift between Australia (Aus) and Antarctica are reconstructed using previously published parameters¹⁴; Antarctica is held fixed. A fourth plate boundary is required by scenario *b*, either between the Pacific and Lord Howe Rise (Lhr), as shown here, or within Antarctica. In *a*, the apparent asymmetry of anomaly 31 with respect to the 2000-m isobath where it crosses the Ross Sea may be due to disruption of the 2000-m contour, and shallowing of the Antarctic continental margin, during later Tertiary extension associated with the Transantarctic Rift. Map is an oblique Mercator projection about 46.40° N, 50.07° W, the revised stage pole predicted for anomalies 25 and 31 on Antarctica west of the Campbell Plateau magnetic bight.

tions called for about 400 km of convergence of these two plates between the times of anomaly 31 and anomaly 25 (ref. 14). If, alternatively, Antarctica had been a single plate during this interval, motion of about 550 km was calculated to have occurred between the Pacific plate and the Lord Howe Rise during the same time period¹⁴. No evidence for such motion has been found in the New Zealand geological record^{30–33}.

The revised Pacific–Antarctica reconstructions, assuming a single Antarctica plate, reduce this discrepancy. They indicate no resolvable displacement of the Pacific plate with respect to the Lord Howe plate between the times of anomalies 31 and 25 (Fig. 5). The uncertainties are too large to resolve less than 200 km of displacement anywhere in the system (either in New Zealand or in Antarctica) during this interval. Within these uncertainties, the revised reconstructions yield a history of motion between the Pacific and Lord Howe plates that is consistent with geological evidence for a lack of early Tertiary deformation in New Zealand and Antarctica.

The apparent overlap in the reconstruction of the Campbell plateau to Antarctica at the time of anomaly 31 (Fig. 6) may be due to a small amount of Tertiary deformation within the Antarctic plate, as discussed above. An additional hint of deformation there comes from the resolvable discrepancy between predicted and observed Australia–Pacific motion in New Zealand during the interval between anomalies 6 and 13 (35.6 to 19.9 Myr). If the Pacific, Antarctica and Australia plates were all rigid during this interval, the plate reconstructions suggest that strike-slip motion along the New Zealand plate boundary began close to 35 Myr, with 450 ± 100 km of right-lateral motion before anomaly 6 time (Fig. 5). The New Zealand geological record, however, is more consistent with slow rifting during Oligocene time^{30,32,33}, changing to major transcurrent motion at approximately the time of the Oligocene–Miocene boundary^{30,33} (23 Myr). This discrepancy could be removed if several hundred kilometres of extension occurred in Antarctica during this period, but the marine data are insufficient to test this idea.

As noted above, the revision of the evolution of the south Pacific proposed here not only yields a more consistent history of deformation in New Zealand than previous reconstructions that ignored the Bellingshausen plate, but also predicts a change in the direction of the Pacific plate with its neighbours that is consistent with the sense and timing of the bend in the Hawaiian-Emperor chain. Using the known history of the Hawaiian-Emperor chain, we show elsewhere²⁵ that reconstructions of the major plates over the hotspots indicate relative migration of the hotspots in the Atlantic and Indian Oceans with respect to the Hawaiian hotspot of 10 to 20 mm yr⁻¹. Moreover, calculations

of motion of the Farallon and Vancouver plates toward North America show a change from rapid (>100 mm yr⁻¹) convergence between the times of anomalies 31 and 18, to slow relative motion parallel to the coast of North America²².

We thank J. Weissel for providing copies of Endeavor magnetic records, Graig McHendrie of the US Geological Survey for assistance with plotting, and R. L. Larson and W. Harbert for constructive criticism of the manuscript. This work was supported by NSF grant OCE-8400090 and by a Fannie and John Hertz Foundation graduate fellowship to J. Stock.

Received 23 July; accepted 9 December 1986.

- Schopf, J. M. *Science* **164**, 63–66 (1969).
- Hamilton, W. *Tectonophysics* **4**, 555–568 (1967).
- Hayes, D. E. & Ringis, J. *Nature* **243**, 86–88 (1973).
- Molnar, P., Atwater, T., Mammerickx, J. & Smith, S. M. *Geophys. J. R. astr. Soc.* **40**, 383–420 (1975).
- Gordon, R. G. & Cox, A. J. *geophys. Res.* **85**, 6534–6546 (1980).
- Suarez, G. & Molnar, P. *J. geophys. Res.* **85**, 5257–5280 (1980).
- Watts, D. R., Watts, G. C. & Bramall, A. M. *Tectonics* **3**, 333–346 (1984).
- Cande, S. C., Herron, E. M. & Hall, B. R. *Earth planet. Sci. Lett.* **57**, 63–74 (1982).
- Engelbreton, D. C., Cox, A. & Gordon, R. G. *J. geophys. Res.* **89**, 10291–10310 (1984).
- Rosa, J. W. C. & Molnar, P. *J. geophys. Res.* (in the press).
- Atwater, T. & Macdonald, K. C. *Nature* **270**, 715–719 (1977).
- Weissel, J. K., Hayes, D. E. & Herron, E. M. *Marine geol.* **25**, 231–277 (1977).
- Hellinger, S. J. *J. geophys. Res.* **86**, 9312–9318 (1981).
- Stock, J. & Molnar, P. *J. geophys. Res.* **87**, 4697–4714 (1982).
- Christoffel, D. A. & Falconer, R. H. K. *Antarctic Res. Ser.* **19**, 197–209 (1970).
- Hayes, D. E., Heirtzler, J. R., Herron, E. M. & Pitman, W. C. III *Prelim. Rep. USNS cruises 22–27, Lamont-Doherty Survey of the World Ocean Vol. 21* (1969).
- Hayes, D. E., Talwani, M., Houtz, R. & Pitman, W. C. III *Prelim. Rep. USNS Eltanin Cruises 28–32, Lamont-Doherty Survey of the World Ocean Vol. 22* (1972).
- Hayes, D. E., Houtz, R., Talwani, M., Watts, A. B., Weissel, J. & Aitken, T. *Prelim. Rep. USNS Eltanin cruises 33–38, Lamont-Doherty Survey of the World Ocean Vol. 23* (1975).
- Berggren, W. A., Kent, D. V., Flynn, J. J. & Van Couvering, J. A. *Geol. Soc. Am. Bull.* **96**, 1407–1418 (1985).
- Kent, D. V. & Gradstein, F. M. *Bull. geol. Soc. Am.* **96**, 1419–1427 (1985).
- Haxby, W. F. *Gravity field of the World's Oceans* (map) (Lamont-Doherty Geological Observatory, Palisades, New York, 1985).
- Stock, J. M. & Molnar, P. (in preparation).
- Stock, J. M. & Molnar, P. *Geology* **11**, 697–701 (1983).
- Molnar, P. & Stock, J. M. *J. geophys. Res.* **90**, 12537–12544 (1985).
- Molnar, P. & Stock, J. M. *Nature* (submitted).
- Clague, D. A. & Dalrymple, G. B. *Geol. Soc. Amer. DNAG Ser.* (in the press).
- Cooper, A. K. & Davey, F. J. *Science* **229**, 1085–1087 (1985).
- Smith, A. G. & Drewry, D. J. *Nature* **309**, 536–538 (1984).
- LeMasurier, W. E. & Rex, D. C. in *Antarctic Earth Science* (eds Oliver, R. O., James, P. R. & Jago, J. B.) 663–670 (Australia Academy of Science, Canberra, 1983).
- Kamp, P. J. J. *Bull. geol. Soc. Am.* **97**, 255–281 (1986).
- Carter, R. M. & Norris, R. J. *Earth planet. Sci. Lett.* **31**, 85–94 (1976).
- Ballance, P. F. *Earth planet. Sci. Lett.* **28**, 356–370 (1976).
- Norris, R. J., Carter, R. M. & Turnbull, I. M. *J. geol. Soc. Lond.* **135**, 191–205 (1978).
- Jurdy, D. M. *Geology* **6**, 469–472 (1979).

A cytosolic protein contains a cryptic mitochondrial targeting signal

Eduard C. Hurt* & Gottfried Schatz

University of Basel, Biocenter, CH-4056 Basel, Switzerland

Cytosolic dihydrofolate reductase from mouse contains a cryptic mitochondrial targeting sequence. If this sequence is attached to the amino terminus of 'passenger' proteins which by themselves cannot enter mitochondria, the resulting fusion proteins are transported into yeast mitochondria.

AMINO-TERMINAL presequences of imported mitochondrial proteins can direct non-mitochondrial passenger proteins into mitochondria¹. These targeting sequences appear to act and fold independently from the attached 'mature' protein^{1,2}. Although mitochondrial presequences do not show significant sequence homology, most of them share overall structural features: they are rich in positively charged and hydroxylated amino acids, devoid of acidic amino acids and show a rather regular alternation between basic and hydrophobic residues. Finally, most mitochondrial presequences analysed so far can potentially form an amphiphilic α -helix with basic and other hydrophilic residues forming a polar face and hydrophobic residues forming the opposite, non-polar face^{3,4}. Amphiphilicity of a presequence may thus be important for protein import into mitochondria³. Artificial presequences composed of only arginine, serine and leucine residues are functionally active and nearly as efficient as authentic mitochondrial presequences⁵. This suggests that the function of a mitochondrial targeting sequence does not depend on a specific primary amino-acid sequence but on overall amino-acid composition: basic and hydrophobic residues and neutral overall hydrophobicity. Such amphiphilic sequences may also occur in many non-mitochondrial proteins. Are these sequences potential mitochondrial targeting signals and, if so, why are they inactive? In this study we have identified a mitochondrial target-

ing signal in cytosolic dihydrofolate reductase from mouse. This signal is masked in the folded enzyme but can be activated if placed in front of appropriate passenger proteins.

Cryptic mitochondrial targeting signal

We looked for cryptic mitochondrial targeting signals in cytosolic dihydrofolate reductase (DHFR) from mouse. As the crystal structure of the highly homologous chicken liver enzyme has been determined to a resolution of 2.9 Å, the positions of α -helical and amphiphilic structures in the folded protein are known⁶. By gene manipulation, the first 85 amino acids of mouse DHFR were fused in-frame to the authentic DHFR enzyme consisting of 187 residues (1–85 DHFR-DHFR; Figs 1 and 2). The resulting fusion protein contained only sequences derived from cytosolic proteins. The amino-terminal 85 residues of DHFR include 14 positively and 7 negatively charged residues as well as 7 serine and threonine residues; the overall hydrophobicity of this sequence is roughly neutral (see ref. 3).

The 1–85 DHFR-DHFR fusion protein and authentic DHFR were expressed *in vitro* by coupled transcription/translation and subsequently incubated with isolated yeast mitochondria (Fig. 2).

Authentic mouse DHFR (relative molecular mass, M_r 21,000 (21 K); Fig. 2, DHFR, lane 1) was neither bound nor imported by de-energized mitochondria (Fig. 2, lanes 2–5). This is expected as a cytosolic protein should not be transported into isolated mitochondria⁷. This is also true *in vivo*: if mouse cytosolic DHFR

* Present address: European Molecular Biology Laboratory, Postfach 1022.09, D-6900 Heidelberg, FRG.

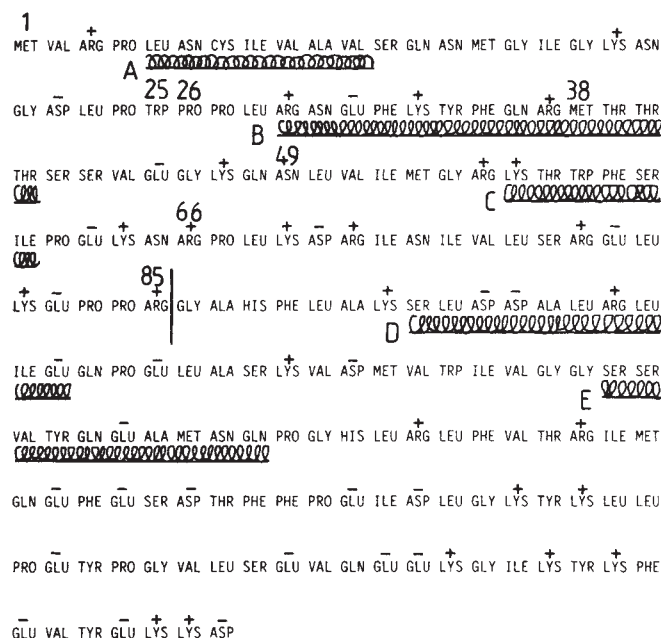


Fig. 1 Primary amino-acid sequence of mouse cytosolic dihydrofolate reductase consisting of 187 residues¹⁴. The five α -helical regions (A-E) occurring in the highly homologous chicken liver enzyme⁶ are shown in coil below the three-letter amino-acid code. Positively and negatively charged amino acids are marked by + and - signs. Residue numbers are given on top of the sequence to indicate the strategy used in dissecting the first 85 amino acids of DHFR for gene fusion studies (for details see Fig. 4).

is expressed in living yeast cells, it stays in the cytosol and is not detectable in mitochondria⁸. However, a protein with completely new properties was created by attaching the amino-terminal 85 amino acids of DHFR in front of intact cytosolic DHFR. First, this fusion protein bound to isolated de-energized mitochondria (Fig. 2, 1-85 DHFR-DHFR, lane 2), but was still on the mitochondrial surface as judged by its protease-sensitivity (lane 3). Upon energization of the mitochondrial membranes, the fusion protein was imported into mitochondria: part of it was protected against externally added proteinase K (lanes 4 and 5). This protected form, however, was degraded by proteinase K when mitochondria were lysed by Triton X-100 before adding the protease (lane 6). Thus, the first 85 amino acids of DHFR transported attached intact DHFR into mitochondria. Similarly, the amino-terminal half of DHFR directed attached subunit IV of cytochrome oxidase lacking most of its own presequence ('pseudo-mature subunit IV') into mitochondria (Fig. 2, 1-85 DHFR mCox IV). Pseudo-mature subunit IV itself is not transported into mitochondria^{5,8}. Quantitation of fluorograms similar to that of Fig. 2 showed that the efficiency of protein import into mitochondria mediated by the first 85 residues of DHFR was about 8-fold lower than that mediated by the authentic subunit IV presequence. Our results show that cytosolic DHFR contains a potential mitochondrial targeting signal that is inactive within the folded protein, but becomes activated if placed in front of suitable passenger proteins.

Authentic mitochondrial presequences most probably fold independently of the attached 'mature' protein^{1,2}. Do the amino-terminal 85 amino acids of DHFR fold independently from the attached intact DHFR moiety in the 1-85 DHFR-DHFR fusion protein? The *in vitro* synthesized, radiolabelled 1-85 DHFR-DHFR fusion protein was incubated with increasing amounts of trypsin (Fig. 3, 1-85 DHFR-DHFR). The intact DHFR enzyme is highly resistant to trypsin⁷, most probably because of its compact folding⁶. Even at the lowest trypsin concentration, a stable breakdown product was formed which co-migrated with authentic DHFR. A similar digestion pattern was observed for a DHFR fusion protein carrying an authentic mitochondrial

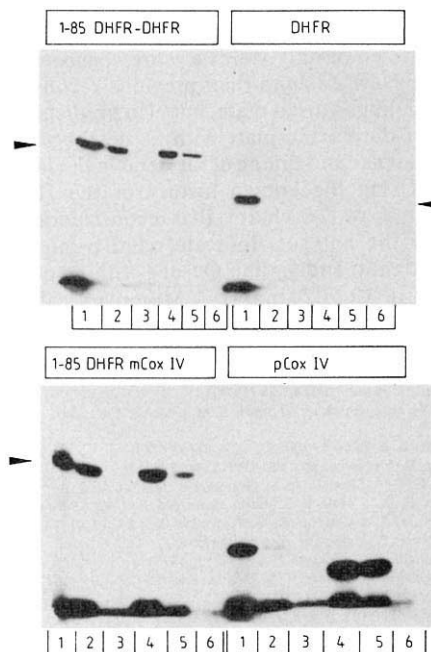


Fig. 2 The first 85 amino acids of cytosolic dihydrofolate reductase transport attached passenger proteins into isolated yeast mitochondria. The protein 1-85 DHFR-DHFR is a fusion protein consisting of the first 85 amino acids of DHFR attached to an intact DHFR molecule; DHFR, intact dihydrofolate reductase; 1-85 DHFR mCox IV, fusion protein consisting of the first 85 amino acids of DHFR attached to pseudomature subunit IV of cytochrome oxidase, lacking most of its authentic presequence; pCox IV, authentic precursor of subunit IV of cytochrome oxidase. The indicated proteins and fusion proteins were synthesized *in vitro* by coupled transcription/translation and incubated with either de-energized or energized yeast mitochondria⁸. After import, mitochondria were reisolated and analysed for bound and imported proteins by SDS-12% polyacrylamide gel electrophoresis and fluorography⁸. Lane 1, translation product obtained by transcription/translation; 10% of the amount added to the mitochondria in the import experiments (lane 2-6) were applied; 2 and 3, binding to mitochondria de-energized by 1 $\mu\text{g ml}^{-1}$ valinomycin; 4, 5 and 6, import into energized mitochondria; the samples in lanes 3 and 5 were treated after binding and import with proteinase K to digest non-imported proteins; the samples in lane 6 were digested with proteinase K in the presence of 1% Triton X-100. Filled arrows, proteins obtained by transcription/translation; open arrow, position of processed, mature subunit IV. For determination of the amount of protein imported into mitochondria, the radiolabelled bands from lanes 1 and 5 were quantified¹⁵; after 20-min incubation, 3% of added 1-85 DHFR-DHFR and 2.7% of 1-85 DHFR mCox IV fusion protein and 20% of authentic subunit IV precursor had been imported into energized mitochondria.

Methods. Fusion genes were constructed as follows. To obtain in-frame fusion between the first 85 amino acids of DHFR and intact DHFR, plasmid pDS5/2-1 (ref. 8), containing the mouse DHFR gene under control of a strong bacteriophage promoter¹⁶ was cut at its single *SacI* site corresponding to amino acid 86 of DHFR. The 3'-protruding ends were made blunt with T4 DNA polymerase, and an *EcoRI* linker (12mer, Biolabs) was attached. It was cut with *EcoRI* and a 270-base-pair fragment corresponding to amino acids 1-85 of DHFR was isolated. This *EcoRI* fragment was inserted into the single *EcoRI* site of plasmid pDS5/2-1. By this gene fusion, the first 85 amino acids of DHFR were joined in-frame to an intact DHFR molecule, to give 1-85 DHFR-DHFR. The amino-acid sequence around the fusion site (which contained eight additional amino acids introduced by gene fusion; Roman numerals) is Arg (residue 85 of DHFR)-Ala(I)-Arg(II)-Asn(III)-Ser(IV)-Gly(V)-Ser(VI)-Gly(VII)-Ile(VIII)-Met(start methionine of the intact DHFR molecule). The 270-base-pair *EcoRI* fragment containing the gene fragment encoding the first 85 amino acids of DHFR was also inserted into the single *EcoRI* site of plasmid pDS5/2-1-pseudo-mature subunit IV⁸. The resulting plasmid was cut with *EcoRV* (corresponding to amino acid 23 of the subunit IV presequence) and partially with *EcoRI* at the 3'-end of the 270-base-pair DHFR insert. After end-filling with the large fragment of *E. coli* DNA polymerase, the plasmid was religated to yield plasmid pDS5/2-1-(1-85)DHFR mCox IV. The amino-acid sequence of the resulting fusion protein (1-85 DHFR mCoxIV) around the fusion site as deduced from the nucleotide sequence is Arg(residue 85 of DHFR)-Ala(I)-Arg(II)-Asn(III)-Tyr(residue 23 of subunit IV presequence)-Leu(24)-Leu(25).

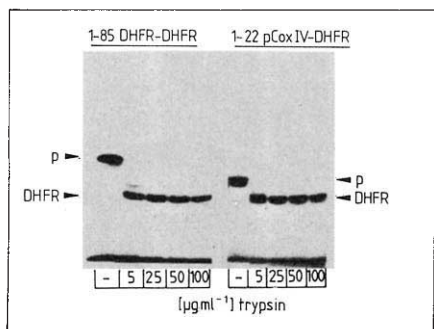


Fig. 3 The 85 amino-terminal residues of DHFR form a separate domain the 1-85 DHFR-DHFR fusion protein. The proteins 1-85 DHFR-DHFR (see Fig. 2) and 1-22 pCox IV-DHFR (a fusion protein consisting of the first 22 amino acids of the cytochrome oxidase subunit IV precursor attached to DHFR¹⁷) were expressed by coupled transcription/translation in the presence of ³⁵S-methionine as described (Fig. 2, legend) and incubated at 0 °C with the indicated amounts of trypsin. Trypsin digestion was stopped after 20 min with 1 mM phenylmethylsulphonylfluoride and the products were analysed by SDS-12% polyacrylamide gel electrophoresis and fluorography. Arrows, positions of the precursor form (p) and of authentic DHFR.

presequence (Fig. 3, 1-22 pCox IV-DHFR). It appears that the intact DHFR moiety within the 1-85 DHFR-DHFR fusion protein exhibits its well-known compact, trypsin-resistant conformation; in contrast, the attached amino-terminal 85 residues, as well as the attached subunit IV presequence, form an exposed domain which is accessible to proteases as well as to the mitochondrial import machinery.

Localization of the targeting sequence

Gene deletion studies have shown that the mitochondrial targeting information is located in distinct parts of a mitochondrial presequence, either at the extreme amino terminus^{1,9} or in the central part of a presequence¹⁰. To localize this cryptic targeting sequence more precisely, the amino-terminal half of DHFR was cut into four pieces using recombinant DNA techniques (see also Fig. 1). We then used an *in vivo* complementation assay⁸ to test which of the four sub-sequences could direct attached pseudo-mature subunit IV into mitochondria. A subunit IV-deficient yeast mutant that no longer assembles an active cytochrome *c* oxidase and thus cannot grow on non-fermentable carbon sources¹¹ was transformed with plasmid-borne fusion genes encoding the various DHFR sequences fused to pseudomature subunit IV. Restoration of growth on a non-fermentable carbon source was taken as proof that the attached

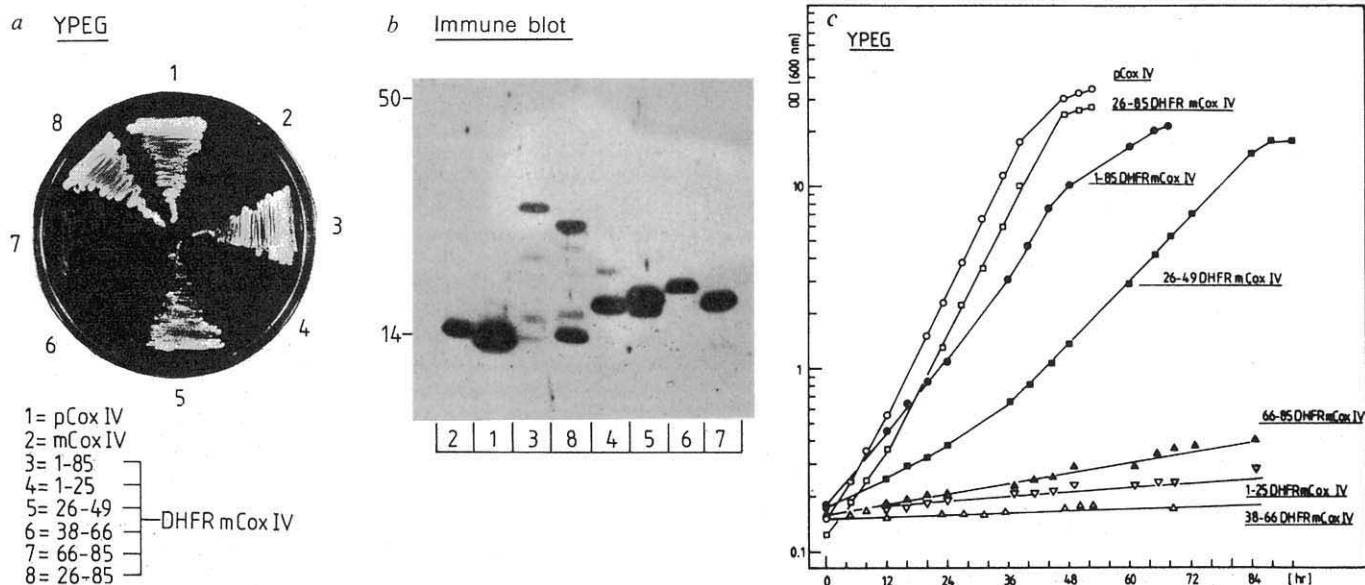


Fig. 4 The cryptic mitochondrial targeting signal in residues 1-85 of DHFR is in residues 26-49. By recombinant DNA techniques, the first 85 amino acids of DHFR were cut into 4 subsequences (residues 1-25, 26-49, 38-66 and 66-85). Each piece was fused in-frame to the amino terminus of cytochrome oxidase subunit IV lacking most of its own presequence and tested by *in vivo* complementation⁸ for its mitochondrial targeting activity. *a*, Growth of transformed cells of the subunit IV-deficient mutant on plates containing glycerol and ethanol as the major carbon and energy source (YPEG-plates). The mutant was transformed with plasmid-borne genes encoding the following proteins: pCox IV, authentic subunit IV precursor; mCox IV, pseudo-mature subunit IV; DHFR mCox IV, fusion proteins containing the indicated DHFR sequences attached to pseudo-mature subunit IV. Transformants were first selected for growth on an uracil-free synthetic medium containing glucose; cells from single colonies were then streaked onto YPEG-plates containing 1% yeast extract, 2% peptone, 3% ethanol and 2% glycerol and allowed to grow for 96 h at 30 °C. *b*, Total protein extracts from the various transformants were analysed by SDS-13% polyacrylamide gel electrophoresis and immune blotting using an antiserum against subunit IV of cytochrome oxidase. Transformants were grown in 2% glucose, 0.67% yeast nitrogen base and 20 μ g ml⁻¹ histidine to an A_{600} of 3, proteins were extracted⁸ and proteins equivalent to $3 \times A_{600}$ were applied onto the gel. The positions of citrate synthase (51K) and of subunit IV of cytochrome *c* oxidase (14K) are indicated on the left. *c*, Growth of transformants in liquid YPEG-medium. The following generation times were calculated for the transformants: pCox IV (3.5 h), mCox IV (no growth); 1-85 DHFR mCox IV (9.5 h, initial phase; 4 h, late phase); 1-25 DHFR mCox IV (>100 h); 26-49 DHFR mCox IV (initial phase 21 h, late phase 11 h); 38-66 DHFR mCox IV (no growth); 66-85 DHFR mCox IV (>70 h); 26-85 DHFR mCox IV (3.5 h).

Methods. To create smaller DNA pieces encoding the various subsequences, the DNA fragment encoding the amino-terminal 85 amino acids of DHFR (Fig. 2) was cut at convenient restriction sites¹⁶ and linker sequences were attached either to the 5'-end (supplying an ATG start codon) or to the 3'-end (to achieve in-frame fusions with the subunit IV sequence). The predicted amino-acid sequences around the various fusion sites are as follows: 1-25 DHFR mCoxIV: Trp(residue 25 of DHFR)-Asn(I)-Ser(II)-Ser(III)-Met(IV)-Ser(residue 20 of the subunit IV presequence); 26-49 DHFR mCoxIV: Met (start methionine)-Pro(residue 26 of DHFR) ... Asn (residue 49 of DHFR)-Ser(I)-Ser(II)-Met(III)-Ser(residue 20 of the subunit IV presequence); 38-66 DHFR mCox IV: Arg(residue 66 of DHFR)-Ala(I)-Arg(II)-Gly(III)-Ser(residue 20 of the subunit IV presequence); 66-85 DHFR mCox IV: Met(residue 1 of DHFR)-Val(2)-Arg(66) ... Arg(85)-Ala(I)-Ala(II)-Asn(III)-Tyr(residue 23 of the subunit IV presequence). All fusion genes were finally cloned as *EcoRI*/*HindIII* fragments into the large fragment of plasmid pAC1 and placed under the control of the yeast alcohol dehydrogenase I promoter, allowing efficient expression of these genes *in vivo*⁸. Plasmids pAC1 containing subunit IV genes with attached DHFR gene fragments were used to transform the subunit IV-deficient mutant WD1 (ref. 11). Transformants were selected on uracil-free plates supplemented with 20 μ g ml⁻¹ histidine and tested for expression of subunit IV and its derivatives by extraction with NaOH, SDS-polyacrylamide gel electrophoresis and immune blotting using an antiserum prepared against subunit IV of yeast cytochrome oxidase. The correct construction of the various fusion genes was ascertained by restriction analysis, DNA sequencing¹⁸ around the fusion site and by detection of the fusion proteins synthesized *in vivo* in total protein extracts prepared from the various transformants.

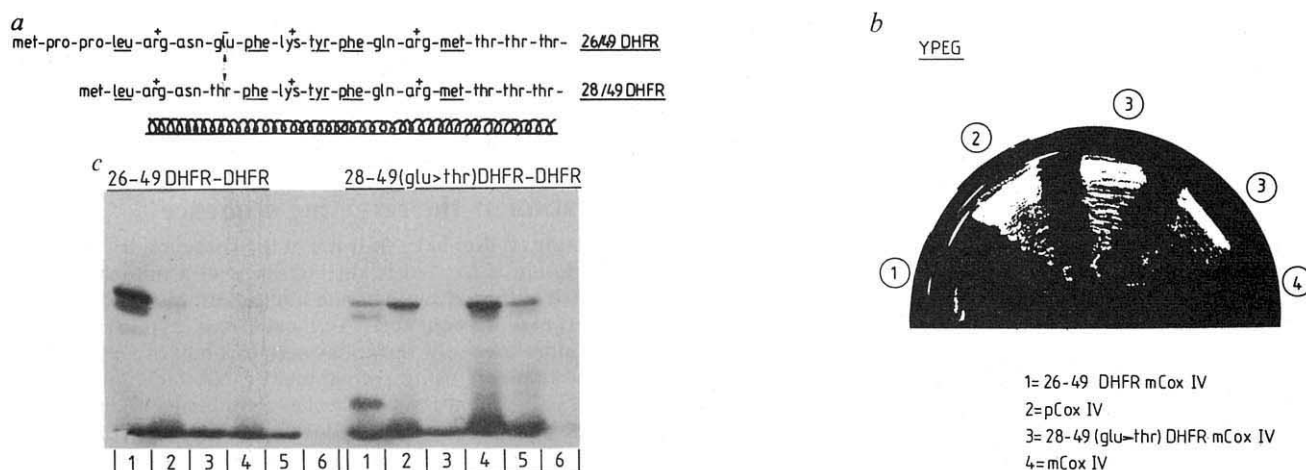


Fig. 5 The targeting efficiency of the cryptic mitochondrial targeting signal within cytosolic DHFR is significantly increased by changing a glutamic acid into a threonine. **a**, Deduced sequence of DHFR residues 26-49 and of the mutated DHFR sequence 28-49. Only the first 17 and 15 amino acids, respectively, are shown. Positively and negatively charged residues are marked by + and -; hydrophobic residues are underlined. The region which forms an α -helix in the folded DHFR is indicated as a coil. Vertical arrows, site of amino-acid change. **b**, Growth on YPEG-plates: the authentic DHFR sequence 26-49 and the mutated sequence 28-49 (glutamic acid 31 changed into a threonine) were attached to pseudo-mature subunit IV (26-49) DHFR mCox IV; 28-49 (Glu $\rightarrow$ Thr) DHFR mCox IV). The corresponding fusion proteins were expressed in the subunit IV-deficient mutant as described (Fig. 4, legend). Transformants were grown for 70 h on YPEG-plates; pCox IV, wild-type subunit IV precursor; mCox IV, pseudo-mature subunit IV. For constructing the mutated DHFR sequence, a DNA duplex was made using a DNA synthesizer (380B, Applied Biosystems) encoding the DHFR sequence 28-33 plus a start methionine. The codon GAG encoding glutamic acid 31 was changed into ACC, encoding threonine. This double-stranded oligonucleotide which contained at the 5'-end *EcoRI* sticky ends and was blunt-ended at the 3'-end (corresponding to the *ScaI* site within the DHFR gene) was inserted into the large *EcoRI/ScaI* fragment of plasmid pDS5/2-1-(26-49) DHFR mCox IV (see Fig. 4, legend) yielding plasmid pDS5/2-1-(28-49) (Glu $\rightarrow$ Thr) DHFR mCox IV. This fusion gene was finally inserted into plasmid pAC1 (see Fig. 4, legend) allowing *in vivo* expression of this fusion protein. **c**, *In vitro* import experiments: for *in vitro* expression of 26-49 DHFR-DHFR and 28-49 (Glu $\rightarrow$ Thr) DHFR-DHFR, the *EcoRI* fragments encoding 26-49 DHFR and 28-49 (Glu $\rightarrow$ Thr) DHFR were excised from the corresponding plasmids and inserted into the *EcoRI* site of plasmid pDS5/2-1. This yielded in-frame fusion between the short DHFR sequences and authentic DHFR. The fusion genes which were under the control of a strong bacteriophage promoter were finally expressed by *in vitro* transcription/translation and tested for import into isolated mitochondria as described (Fig. 2, legend) Lane 1, 10% of the total amount of *in vitro* translation products which were added to mitochondria; 2, binding to de-energized mitochondria; 3, de-energized mitochondria plus proteinase K; 4, import into energized mitochondria; 5, energized mitochondria plus proteinase K; 6, energized mitochondria plus proteinase K and Triton X-100.

DHFR sequence contained a mitochondrial targeting signal. Growth was tested on solid (Fig. 4a) or liquid medium (Fig. 4c) containing ethanol and glycerol as non-fermentable carbon sources. As a control, total protein extracts prepared from the various transformants grown in glucose-containing medium were analysed by immune blotting to confirm that DHFR-subunit IV fusion proteins of the expected size were indeed made (Fig. 4b). The *in vivo* complementation approach confirmed the earlier conclusion (Fig. 2) that the first 85 amino acids of the DHFR contain a potential mitochondrial targeting sequence (Fig. 4a, 3). However, restoration of growth on glycerol was only partial; the targeting efficiency of the first 85 residues of DHFR is thus lower than that of the authentic subunit IV presequence, in agreement with the *in vitro* import data (Fig. 2). Upon dissecting the amino-terminal half of DHFR, only residues 26-49 retained some mitochondrial targeting function; residues 1-25, 38-66 and 66-85 were almost completely inactive (Fig. 4a,c). Only part of the targeting information initially present in residues 1-85 was recovered in sequence 26-49 (Fig. 4c; compare growth rates of 1-85 DHFR mCox IV with 26-49 DHFR mCox IV). In contrast, all the targeting information present in residues 1-85 appeared to be retained in the sequence 26-85: the corresponding yeast transformant grew as fast as the wild-type strain in YPEG-medium (Fig. 4c). In conclusion, a cryptic mitochondrial targeting sequence was localized in the DHFR sequence 26-49.

Residues 26-49 of DHFR resemble a mitochondrial pre-sequence (Fig. 1 and 5a): they include four positively charged amino acids and their amino-terminal half can potentially form an amphiphilic α -helix (see also discussion). However, two negatively charged residues are also present in this sequence. Because acidic residues are only rarely found in natural mitochondrial presequences^{1,4}, we suspected that the negative charges could interfere with the mitochondrial targeting function of this sequence. In an attempt to eliminate this interference, we used site-directed mutagenesis to convert glutamic acid 31

(this residue is located in a region which can potentially form an amphiphilic α -helix) into a threonine; we also removed the two proline residues 26 and 27 to give only an α -helical region at the amino-terminus of the fusion protein (Fig. 5a). *In vivo* complementation showed that these modifications did indeed increase mitochondrial targeting. The subunit IV-deficient yeast mutant expressing the mutated DHFR sequence 28-49 attached to pseudo-mature subunit IV grew as fast as the wild-type strain (Fig. 5b). In addition, when the mutated targeting sequence was attached to the amino terminus of authentic DHFR, import of the *in vitro*-synthesized fusion protein into isolated mitochondria was much more efficient than that of the original 26-49 DHFR-DHFR fusion protein (Fig. 5c). Approximately 12% of the modified fusion protein was imported, which is comparable to other DHFR fusion proteins carrying authentic mitochondrial presequences.

Discussion

We show here that a cytosolic protein contains a potential mitochondrial targeting sequence. This result is remarkable for several reasons. First, it makes it very likely that potential mitochondrial targeting sequences are hidden in many non-mitochondrial proteins. Our *in vivo* complementation approach makes it easy to track down such cryptic mitochondrial targeting sequences. Second, the result does not explain why mitochondrial targeting sequences within non-mitochondrial proteins are non-functional. We cannot exclude that a small number of these non-mitochondrial proteins slip into mitochondria, but significant missorting does not occur. For example, mouse cytosolic DHFR is not imported into mitochondria and is exclusively found in the cytosol even when expressed in large amounts in living yeast cells⁸.

What prevents proteins with cryptic mitochondrial targeting sequences from being transported into mitochondria? We propose that a mitochondrial targeting sequence must fulfill two

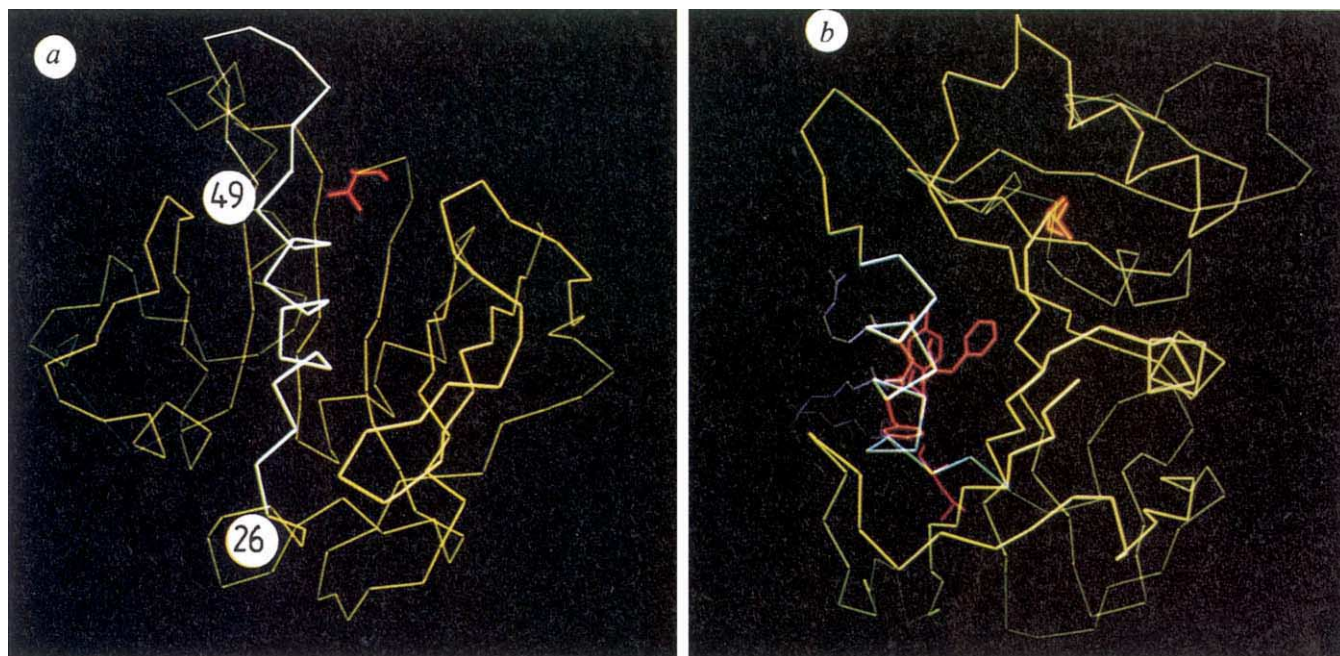


Fig. 6 Crystal structure of chicken liver dihydrofolate reductase (α -carbon backbone folding)⁶. *a*, The DHFR sequence 26–49, containing a potential mitochondrial targeting signal is shown in white; the start of the DHFR sequence is shown in red. *b*, The amphiphilic α -helix B (see Fig. 1) between residues 29 and 41 is shown in white. Three positively charged residues (violet) face out of the molecule and three hydrophobic residues (red) face towards the interior of the enzyme. The Tyr residue corresponding to Phe33 of the mouse enzyme has been changed into a Phe residue to represent the situation in the mouse enzyme.

criteria to be active: it must fold into an 'import-competent' (presumably amphiphilic³) structure, and it must be exposed on the surface of the precursor protein. According to this model, the targeting sequence in residues 1–85 of DHFR is cryptic because it meets the first, but not the second of these criteria; the segment from residues 29–41 is an amphiphilic helix in the folded enzyme, but its hydrophobic face is directed towards the protein's interior and should not be able to interact with the mitochondrial surface (Fig. 6). Our model could also explain why mitochondrial targeting sequences are usually located at the amino-terminal end of precursor proteins. Such a location allows the targeting sequence to fold into an independent, import-competent domain without compromising the stable folding of the attached 'mature' polypeptide chain. Conversely, the model predicts that functional mitochondrial targeting sequences should frequently become cryptic if artificially placed into an internal location. We found that the presequence of cytochrome oxidase subunit IV was rendered inactive if placed between two subunit IV proteins lacking most of their own presequence (unpublished data).

Our attempts to pinpoint the mitochondrial targeting sequence in residues 1–85 of DHFR were only partly successful. The signal was clearly not located between residues 1–25 and was fully recovered within residues 26–85. However, further subdivision of that latter region yielded only partial recovery of the signal. This could reflect an inappropriate choice of cutting sites (for instance, cutting within the targeting sequence) or cooperation between the neighbouring sub-sequences. It has been shown that identical pentapeptide sequences can assume different secondary structures in different proteins¹². The function of the DHFR-derived mitochondrial targeting sequence

might thus be affected by neighbouring contiguous sequences.

It could be argued that the complementing efficiencies of the various DHFR sequences do not reflect true import efficiencies, but the abilities of the various fusion proteins to assemble into an active cytochrome oxidase molecule. Although this cannot be rigorously excluded, it appears very unlikely. As shown elsewhere⁸, subunit IV tolerates extensive modifications of its amino terminus without losing its ability to assemble into an active holoenzyme. Similarly, 1–85 DHFR mCox IV complements better than most of the other fusion proteins described here even though it carries the longest amino-terminal extension and even though this extension is removed only very inefficiently *in vivo* (Fig. 5).

Our present finding shows that a potential mitochondrial targeting sequence occurs within a non-mitochondrial protein. Additional evidence from our laboratory¹³ has revealed that such cryptic sequences can be recovered with relatively high frequency from random fragments of total *E. coli* DNA as well as from different fragments of the mouse DHFR gene read in various reading frames or in the opposite orientation. As discussed more fully elsewhere¹³, such DNA sequences encoding cryptic mitochondrial targeting signals may have served as a source of mitochondrial presequences during the evolution of mitochondria from endosymbionts.

This work was supported by grants 3.394–0.83, 3.660–0.84 and 3.335.0.86 from the Swiss NSF. We thank Drs H. Bujard and D. Stueber for plasmid pDS5/2, Dr J. Kraut for sending us the unpublished crystallographic coordinates of chicken liver DHFR and for allowing us to show the three-dimensional structure of chicken liver DHFR, U. Müller for technical assistance and M. Probst for typing the manuscript.

Received 9 October; accepted 4 December 1986.

- Hurt, E. C. & van Loon, A. P. G. M. *Trends Biochem. Sci.* **11**, 204–207 (1986).
- Eilers, M. & Schatz, G. *Nature* **322**, 228–232 (1986).
- Roise, D., Horvath, S. J., Tomich, J. M., Richards, J. H. & Schatz, G. *EMBO J.* **5**, 1327–1334 (1986).
- von Heijne, G. *EMBO J.* **5**, 1335–1342 (1986).
- Allison, D. & Schatz, G. *Proc. natn. Acad. Sci. U.S.A.* **83**, 9011–9015 (1986).
- Volz, K. W. *et al. J. Biol. Chem.* **257**, 2528–2536 (1982).
- Hurt, E. C., Pesold-Hurt, B. & Schatz, G. *EMBO J.* **3**, 3149–3156 (1984).
- Hurt, E. C., Müller, U. & Schatz, G. *EMBO J.* **4**, 3509–3518 (1985).
- Hurt, E. C., Pesold-Hurt, B., Suda, K., Oppliger, W. & Schatz, G. *EMBO J.* **4**, 2061–2068 (1985).
- Horwich, A. L., Kalousek, F., Fento, W. A., Pollock, R. A. & Rosenberg, L. E. *Cell* **44**, 451–459 (1986).
- Dowhan, W., Bibus, C. R. & Schatz, G. *EMBO J.* **4**, 179–184 (1985).
- Kabsch, W. & Sander, C. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1075–1078 (1984).
- Baker, A. & Schatz, G. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Stone, D. & Phillips, A. W. *FEBS Lett.* **74**, 85–87 (1986).
- Suissa, M. *Analyt. Biochem.* **133**, 511–514 (1983).
- Stueber, D., Ibrahim, I., Cutler, D., Dobberstein, B. & Bujard, H. *EMBO J.* **3**, 3143–3148 (1984).
- Hurt, E. C., Pesold-Hurt, B. & Schatz, G. *FEBS Lett.* **178**, 306–310 (1984).
- Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).

Detached nuclear-like activity in the radio galaxy PKS 2152-69

C. N. Tadhunter*†, R. A. E. Fosbury‡, L. Binette§, I. J. Danziger§ & A. Robinson§

* Royal Greenwich Observatory, Herstmonceux Castle, Hailsham, East Sussex BN27 1RP, UK and Astronomy Centre, University of Sussex

‡ Space Telescope-European Coordinating Facility, European Southern Observatory, Karl-Schwarzschild-Straße-2, D-8046 Garching bei München, FRG

§ European Southern Observatory, La Silla, Chile

The effects of nuclear activity in galaxies are known to extend well beyond the immediate nuclear environment. This is particularly true in some quasars and the active ellipticals or radio galaxies where the influence of the central engine can be apparent on scales of up to several megaparsecs. In addition to the large-scale regions of non-thermal radio emission there are sometimes sources of optical, both line and continuum, emission which appear to be associated in some way with the transport of energy from the nucleus to the larger structures. This letter reports the discovery of a bright cloud of gas at a projected distance of 8 kpc ($H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ used throughout) and approximately along the radio axis of the radio galaxy PKS 2152-69. It is shown to radiate a nebular spectrum including lines from the highly ionized species He^+ , Ca^{4+} , Fe^{6+} and Fe^{9+} . A compact source of optical continuum radiation within the cloud has an energy distribution which is considerably bluer than the residual light from the underlying galaxy.

The presence of regions of ionized gas at distances ranging from a few to a hundred or so kiloparsecs (extended emission line regions—EELRs) from the nuclei of a significant fraction of radio galaxies and quasars is now well established and the current status of the observational studies has been reviewed recently in refs 1-3. While in some cases, notably in 3C 277.3 (refs 4,5), there is a clear connection between this line emission and radio jets, this may not always be the case and there is, as yet, no satisfactory explanation for the mechanism of ionization of the gas and its resulting physical state. If such an understanding could be gained, the observations of these regions would, in addition to the picture they are already giving us of the interaction between the nucleus and its environment, provide valuable information about the origin, composition and dynamical state of the interstellar medium at large distances from the host galaxies.

The nuclear and radio properties of the relatively nearby radio galaxy PKS 2152-69 have been discussed in refs 7-14 (see also Table 1). A more extensive set of data, addressing aspects in addition to the highly ionized cloud (HIC) and including new radio and ultraviolet observations of the galaxy and its environment is being presented elsewhere¹⁵. While the HIC is clearly associated with the luminous elliptical galaxy in both position and velocity, it exhibits several characteristics which are remarkably reminiscent of those shown by active nuclei themselves. These similarities may extend to less extreme examples of the extended emission phenomenon. Studies of these extranuclear active regions could therefore offer possibilities to investigate some of the physical mechanisms which operate in the nuclear regions but in an environment which is less complex and confusing. In addition, if the origin of this excited gas in active ellipticals is the cooling flow seen in a number of clusters and relatively nearby early-type galaxies (see for example, ref. 16), then the active objects provide a means to investigate some aspects of this phenomenon in a way which complements the X-ray observations.

The spectrophotometric and imaging observations reported here were carried out respectively with the ESO 3.6 m and ESO/Max-Planck Institute for Astrophysics 2.2 m telescopes on La Silla, Chile. In all cases, the detectors were RCA (Type SID 501) back-illuminated CCDs (charge-coupled devices). The images were taken with interference filters with FWHM (full width at half maximum) of 80 Å centred at $\lambda = 6,770 \text{ Å}$ for the on-band and $\lambda = 6,607 \text{ Å}$ for the off-band exposures of 30 and 20 min respectively. At the $f/8$ focus, a CCD pixel subtends 0.36 arc s. The spectra taken with the Boller and Chivens spectrograph have a pixel size in the spatial direction of 1.15 arc s and cover a spectral range of 4,600-7,300 Å and 3,700-6,400 Å with a spectral resolution (FWHM) of approximately 13 Å. For the ESO faint object spectrograph and camera data¹⁷, the corresponding figures are 0.675 arc s, 3,800-7,000 Å and 20 Å. The slit widths varied from 2.5 to 4.0 arc s and the slit was oriented in several position angles on the sky: radially and tangentially with respect to the nucleus—HIC vector and also, for the observations which extend further to the blue, along the local vertical to minimize the effects of atmospheric dispersion.

The data reduction, including bias subtraction, flat-fielding, sky-subtraction and wavelength (for the spectroscopy), atmospheric extinction and flux calibration were carried out using the UK STARLINK SPICA spectral reduction software and the ESO IHAP image reduction system. The flux calibrations are based on observations of white dwarfs from ref. 18. The on- and off-band images were scaled and registered to allow subtraction to produce a continuum-free $\text{H}\alpha + [\text{NII}]$ emission map.

The distribution of ionized gas in PKS 2152-69 can be seen in Fig. 1 which shows the continuum and $\text{H}\alpha + [\text{NII}]$ emission line maps from the imaging CCD data. Three components of line emission can be recognized and their corresponding spectra are reproduced in Fig. 2. (1) The bright, unresolved nucleus which has a spectrum of intermediate ionization with broad wings to both permitted and forbidden lines. (2) A low ionization filamentary component (LIF) having a radial structure together with a more symmetric 'open spiral' appearance with 'arms' northwest and southeast of the nucleus. This is reminiscent of the cooling flow filaments seen in some ellipticals and cluster cores (ref. 16). (3) The bright HIC with a fan-shaped structure, 10 arc s to the northeast of the nucleus.

Emission line fluxes derived from the spectra shown in Fig. 2 are given in Table 2. The uncertainty in these values is typically <10% for strong unblended lines and <30% for strong blended lines or weak isolated lines: greater uncertainties are indicated in the table by a colon.

In Fig. 2c, the HIC spectrum is from an aperture which includes most of the emission and therefore represents an average over the region. It is clear from our spectra, however, that the observations with the slit in the radial and tangential directions both show considerable ionization gradients within the cloud. In the radial direction, $\text{HeII}\lambda 4,686/\text{H}\beta$ decreases from 0.71 closest to the nucleus to 0.37 on the outer edge of the cloud while a representative low ionization ratio, $[\text{NII}]\lambda 6,583/\text{H}\alpha$, increases from 0.22 to 0.55. In the tangential direction also, the high ionization lines peak along the central

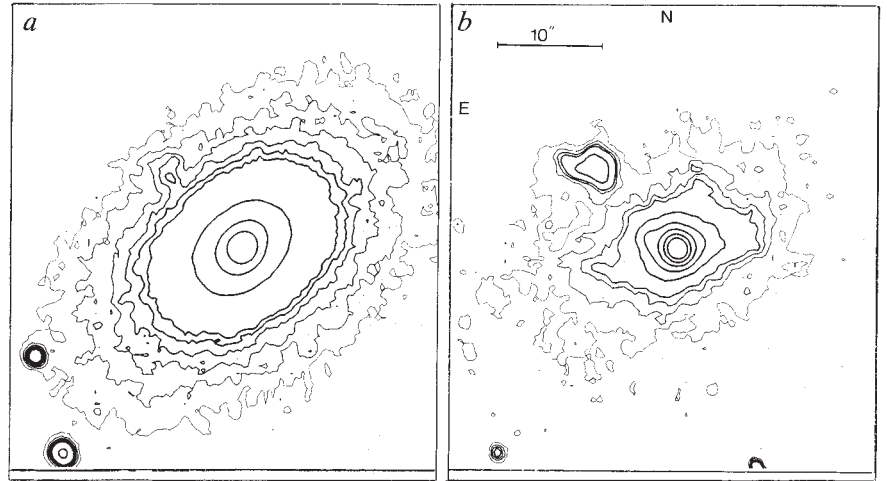
Table 1 Optical and radio properties of PKS 2152-69

Optical		Radio	
V	13.70	$S_{1.4\text{GHz}}$	22.7 Jy
B-V	1.044	$\alpha(f_\alpha \propto \nu^\alpha)$	-0.7
U-B	0.15		
z	0.0282		
Major axis PA	$135^\circ \pm 5^\circ$	Radio axis PA	$23^\circ \pm 3^\circ$
M_B	-21.5	$P_{1.4\text{GHz}}^{\text{tot}}$	$8.2 \times 10^{25} \text{ W Hz}^{-1}$

The optical photometry was by Fosbury in September 1978 with the ESO 1.0 m telescope using a 62 arc s aperture and the radio flux is from ref. 6 after subtracting the unrelated eastern component. The radio axis PA is measured from new maps (Tadhunter *et al.*, in preparation).

† Present address: University of St Andrews, University Observatory, Buchanan Gardens, Fife KY16 9LZ, UK.

Fig. 1 CCD images of PKS 2152–69: *a*, Off band (continuum), *b*, On band line ($H\alpha + [NII]$) minus continuum. The contour levels are chosen to reveal the low brightness features and are logarithmically spaced above about 10% of the sky level.



axis while those of low ionization peak at the edges. The state of ionization is highest at the side of the cloud facing the nucleus where the spectrum shows remarkably strong lines of [Cav], [FeVII] and [FeX]: this is illustrated in Fig. 2*d*. At this location, there is also an apparently featureless continuum with an energy distribution which is considerably bluer than the underlying galaxy; evidence for this source can be seen in the off-band image (Fig. 1*a*). While our spectral coverage is too restricted to determine the shape of this continuum, it has a flux at 5,000 Å, after the subtraction of the galaxy continuum determined from the profile in the tangential direction, of $17 \pm 4 \mu Jy$.

The existing radio maps do not allow us to draw detailed conclusions about the relationship between the radio and optical structures but it is clear^{8,11} (Table 1), at least, that the radio axis and the HIC lie in the same (NE) quadrant.

The velocity field of the ionized gas in PKS 2152–69 is chaotic with no definite large-scale pattern. We can limit the mean radial velocity differences for the HIC to $<100 \text{ km s}^{-1}$ and for the LIF to $<400 \text{ km s}^{-1}$ with respect to the peak velocity of the nuclear gas. The HIC does, however, exhibit a velocity gradient of 400 km s^{-1} in the tangential slit data. Also, in higher spectral resolution data the [OIII] $\lambda\lambda 4959, 5007$ lines show a faint wing extending more than $2,000 \text{ km s}^{-1}$ towards the blue. There is also a fainter cloud some 25 arcs to the north-east of the

nucleus along the same position angle as the HIC which has the much higher velocity of $+700 \text{ km s}^{-1}$. Its relationship to the other components is not yet clear but, since on the spectra it is detected only in the [OIII] lines, the ionization state must be high, closer to the HIC than to the LIF.

The emission line spectrum of the HIC is similar to some of the most highly ionized galactic nuclei known, such as III Zw 77 and Tololo 0109–383^{19,20} which are thought to be photoionized by a relatively hard EUV (extreme ultraviolet) spectrum. There is further evidence for the predominance of photoionization over collisional ionization processes in this and in other EELRs. The electron temperature, measured from the [OIII] line ratio assuming a low electron density, of $16,000 \pm 4,000 \text{ K}$ is considerably lower than the $\approx 60,000 \text{ K}$ predicted by shock heating models which produce a similar [OIII]/H β ratio (see Table 2). Such shock models also predict much stronger lines of [OII] and [SII] than we observe.

The presence of marked ionization gradients across the HIC and the existence of the blue continuum source at the peak of this ionization structure suggests that the photoionization source is local to the cloud. An alternative hypothesis of ionization by the dilute radiation field from the nucleus of the parent galaxy is difficult to reconcile with the observations in several respects. If the nucleus radiates isotropically, the existence of the low

Table 2 Emission line fluxes in PKS 2152–69

Line	Nucleus	Observed line intensities		Shock	Models Phot. 1	Phot. 2
		LIF	HIC (av.)			
3,727 [OII]			181	1,780	621	438
3,869 [NeIII]			53	115	88	68
4,340 H γ			48			
4,363 [OIII]			21	89	5	5
4,686 HeII			56	47	34	51
4,861 H β	100	100	100	100	100	100
5,007 [OIII]	872	110	895	843	1,010	867
5,200 [NI]	51			20	4	3
5,309 [Cav]			6:			
5,721 [FeVII]			13			
5,876 HeI	18		6:	15	11	9
6,087 [FeVII]+[Cav]			15			
6,300 [OI]	316	52	21	36	28	19
6,373 [FeX]			14:			
6,562 H α	1,830	248	349	306	288	287
6,583 [NII]	blended H α	278	141	191	178	125
6,725 [SII]	325	263	103	537	77	54
7,135 [AIII]	33:		21	29	14	10
7,325 [OII]	107:			90	10	8
$F_{H\beta} (10^{-16} \text{ erg cm}^{-2} \text{ s}^{-1})$	96	3	23	—	—	—

The observed values are for the regions described in Fig. 2. In the last three columns are shown, for comparison, the predictions of three models: Shock-shock model with $V_s = 164 \text{ km s}^{-1}$, Phot. 1—ionization bounded photoionization model with high temperature black body ionizing spectrum, $T_b = 1.3 \times 10^5 \text{ K}$, $U = 1.5 \times 10^{-3}$; Phot. 2—composite photoionization model, average over radial direction.

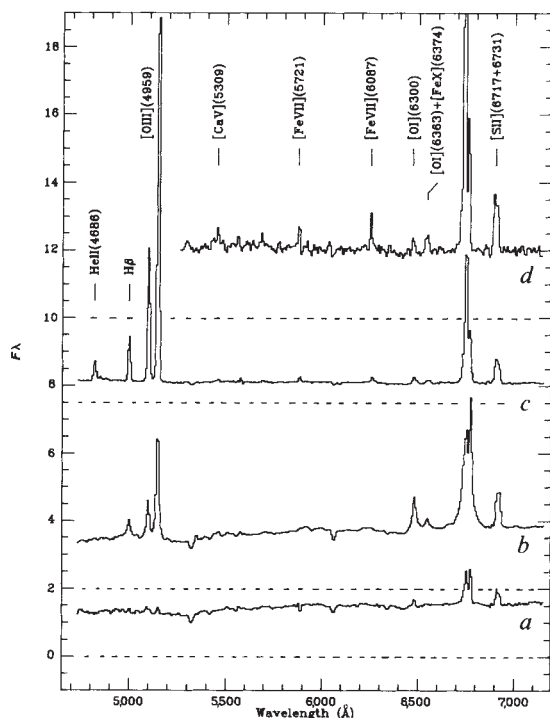


Fig. 2 Optical spectra of the three components of ionized gas in PKS 2512-69: *a*, the low ionization filaments (from a region 4.6×2.5 arc s PA = 290° , 8.6 arc s east of the nucleus; scale $\times 1$). *b*, The nucleus (aperture: 6.6×2.0 arc s PA = 45° ; scale $\times 0.1$). *c*, The high ionization cloud (aperture: 4.6×4.0 arc s PA = 315° , centred 10.9 arc s northeast of the nucleus; scale $\times 1$). *d*, The highly excited part of the HIC coincident with the blue continuum source (aperture: 1.2×2.0 arc s PA = 315° , scale $\times 10$).

ionization filamentary structure around and closer to it than the HIC but having a lower surface brightness would then be inconsistent with the relative ionization states of the three components. Also, we find no evidence for a non-stellar component in the nuclear continuum which, when extrapolated into the ultraviolet assuming a power law spectrum, would provide sufficient ionizing photons into the small solid angle subtended by the HIC. If the nuclear radiation field were anisotropic, it would need to be very highly collimated with an opening angle of $\leq 5^\circ$ in the direction of the HIC in order to account for the observed ionization structures.

Photoionization models using solar abundances and power law continua characterized by values of the spectral index $-1.5 \leq \alpha \leq -1$ reproduce with reasonable accuracy the line spectra of narrow line active galactic nuclei^{21,22} (the models presented here were computed using a multi-purpose code²³ which calculates the line emissivities radiated by a photoionized or collisionally ionized gas) although the origin of the more highly ionized species remains an open question. There are several constraints on such a model for the HIC. The extrapolation of a power law continuum which has sufficient ionizing photons to account for the observed H β luminosity must not give a flux at optical wavelengths greater than that observed. This condition requires that $\alpha \geq -1$; the limit corresponding to complete absorption of all ionizing photons. In photoionization models, a power law of $\alpha \approx -1$ which extends to high energies produces an emission spectrum with very strong lines of [OI] and other neutral and singly ionized species due to the creation and heating by X rays of an extended semi-ionized region beyond the HII zone^{24,25}. The [OI] strength can be reduced to that observed by reducing the fraction of high energy photons in the ionizing continuum. As steeper power law slopes are ruled out by the optical continuum limit, this can only be done by truncating a flat power law spectrum. Photoionization models with $\alpha = -1$ and a high energy cutoff at 155 eV reproduce the majority of the measured

emission line strengths with reasonable accuracy but it must be noted that the alternative hypothesis of a black-body spectrum with a temperature $T_b = 1.3 \times 10^5$ K produces a line spectrum which is essentially identical to that of this truncated power law.

The changes in the ratios of [OII] $\lambda 5,007$, [OI] $\lambda 6,300$, [NII] $\lambda 6,583$ and HeII $\lambda 4,686$ to H β from the front to the rear of the cloud (see Tadhunter *et al.* in preparation) can be modelled well by combining a high ionization, matter bounded component with a low ionization, optically thick component and keeping the respective ionization parameters ($U = F_0/cn_H$ where F_0 is the incident photon flux and n_H is the hydrogen number density) fixed, increasing the relative contribution of the latter towards the outer part of the cloud. The spatially averaged results of such a model, again using a black-body with $T_b = 1.3 \times 10^5$ K are given in the last column of Table 2. The weakness of the observed [OII] $\lambda 3,727$ is a puzzle, even in the context of the photoionization models, and may have to be attributed to collisional deexcitation resulting from electron densities of approximately 10^3 cm^{-3} or greater.

Neither the truncated power law nor the thermal continuum extend to high enough energies to produce by photoionization the line of [FeX] which is observed although it may be possible to produce the [CaV] and [FeVII] in this way. A thermal origin for these ions may be required in our picture. We note that, although such lines are seen relatively frequently in active galactic nuclei, it is not yet clear whether they are produced by photo- or collisional ionization^{20,26}. It may be that these species have an origin which is intimately associated with the source of the ionizing continuum since they appear here to be spatially coincident.

The question of the nature of the ionizing source in the HIC is difficult to answer until higher resolution radio maps become available at this southern declination. The most likely explanation at present appears to be the type of jet-cloud interaction described in the case of 3C277.3 (ref. 4). The hypothesis that the HIC is simply an independent active galaxy in its own right cannot be entirely ruled out although the approximate coincidence with the radio axis of the elliptical, the presence of a more distant highly ionized cloud along the same axis and the asymmetric position of the continuum source within the cloud all appear to favour the interaction idea. Alternatively, a high excitation starburst, perhaps triggered by the radio jet-ISM interaction in the manner suggested to explain Minkowski's object²⁷ is an hypothesis with some attractions but the extreme state of ionization is in marked contrast both with this example and with the known starburst nuclei.

A comparison of the HIC, the LIF and spectra of ionized gas around other radio galaxies with those of narrow line galactic nuclei show marked similarities which can be explained, in the context of our photoionization modelling, if the shape of the ionizing spectrum is similar in the nuclei and in the EELRs but there is a wide range in the effective dilution of the ionizing radiation in both the nuclei and the extended gas (Robinson *et al.*, in preparation). This suggests that the physical mechanism which generates photoionizing radiation in some galactic nuclei, particularly the narrow line radio galaxies, may be capable of operating at a considerable distance from the nucleus itself. The highly collimated jets which power the outer radio structures appear to be the most suitable choice for the source of energy while the cooling flow, evidence for which is seen in the LIF, may be the source of the gas.

This work is based partly on observations made at the ESG, La Silla. We thank Paola Focardi for obtaining and reducing some of the CCD images used in this investigation. C.N.T. thanks the SERC for a research studentship, R.A.E.F. is affiliated to the Astrophysics Division, Space Science Department, European Space Agency and A.R. is a Royal Society fellow.

Received 20 October 1986; accepted 9 December 1986.

1. Stockton, A. Review paper presented at the third Asian-Pacific regional meeting of the IAU, Kyoto, Japan (1985).

2. Fosbury, R. A. E. in *Structure and Evolution of Active Galactic Nuclei*, Trieste, April 10-13, 1985 (ed. Guiricín, G. et al.) 297-308 (Reidel, Dordrecht, 1986).
3. van Breugel, W. *Can. J. Phys.* **64**, 392-397 (1986).
4. van Breugel, W., Miley, G., Heckman, T. M., Butcher, H. & Bridle, A. *Astrophys. J.* **290**, 496-516 (1985).
5. Miley, G., Heckman, T. M., Butcher, H. & van Breugel, W. *Astrophys. J.* **247**, L5-L9 (1981).
6. Ekers, J. A. (ed.), *Aust. J. Phys. Suppl.* **7**, (1969).
7. Westerlund, B. E. & Wall, J. V. *Astron. J.* **74**, 335-351 (1969).
8. Schwarz, U. J., Cole, D. J. & Morris, D. *Aust. J. Phys.* **26**, 661-673 (1973).
9. Schilizzi, R. T. & McAdam, W. B. *Mem. R. astr. Soc.* **79**, 1-73 (1975).
10. Schilizzi, R. T. *Mem. R. astr. Soc.* **79**, 75-100 (1975).
11. Christiansen, W. N. et al. *Mon. Not. R. astr. Soc.* **181**, 183-202 (1977).
12. Marenbach, G. & Appenzeller, I. *Astron. Astrophys.* **108**, 95-98 (1982).
13. Danziger, I. J. & Goss, W. M. *Mon. Not. R. astr. Soc.* **202**, 703-715 (1983).
14. Whittle, M. *Mon. Not. R. astr. Soc.* **213**, 1-31 (1985).
15. Tadhunter, C. N. thesis, Univ. Sussex (1986).
16. Fabian, A. C., Nulsen, P. E. J. & Canizares, C. R. *Nature* **310**, 733-740 (1984).
17. Buzzoni, B. et al. *ESO Messenger*, No. **38**, 9-13 (1984).
18. Oke, J. B. *Astrophys. J. Suppl. Ser.* **27**, 21-35 (1974).
19. Osterbrock, D. E. *Astrophys. J.* **246**, 696-707 (1981).
20. Fosbury, R. A. E. & Sansom, A. E. *Mon. Not. R. astr. Soc.* **204**, 1231-1236 (1983).
21. Stasińska, G. *Astron. Astrophys.* **135**, 341-355 (1984).
22. Ferland, G. J. & Osterbrock, D. E. *Astrophys. J.* **300**, 658-668 (1986).
23. Binette, L. A., Dopita, M. A. & Tuohy, I. R. *Astrophys. J.* **297**, 476-491 (1985).
24. Kwan, J. & Krolik, J. H. *Astrophys. J.* **233**, L91-L95 (1979).
25. Weisheit, J. C., Shields, G. A. & Tarter, C. B. *Astrophys. J.* **245**, 406-415 (1981).
26. Penston, M. V., Fosbury, R. A. E., Boksenberg, A., Ward, M. J. & Wilson, A. S. *Mon. Not. R. astr. Soc.* **208**, 347-364 (1984).
27. van Breugel, W., Filippenko, A. V., Heckman, T. M. & Miley, G. *Astrophys. J.* **293**, 83-93 (1985).

Marginal gravitational lenses of large separation: probing superclusters

Israel Kovner*

Astrophysics Department, Princeton University, Princeton, New Jersey 08544, USA

The surface mass density (Σ) of clusters of galaxies and superclusters is normally too low to split images¹⁻⁵, even for a large source redshift (z_s) and an optimal lens redshift (z_l). Because the distribution function of Σ decreases steeply with Σ , a cluster or supercluster found responsible for multiple imaging has a high probability of being a marginal lens⁵. Such a lens, barely able to split images of a distant source, has a subcritical surface mass density and remarkable properties unlike those of lenses with a density above critical. As I show here, these properties can easily be recognized observationally when the image separation is larger than is normal for a lens made of a galaxy: (1) there may be no prominent excess of galaxies and no microwave background inhomogeneities in the immediate area of the images, (2) there may be an enhancement of the number of observable quasars at very different redshifts in an area much larger than the image separation, (3) there may be split images in the vicinity of unsplit images of other sources of even larger redshifts. If split images of the same source separated by many arc seconds are observed and the responsible lens is a non-exotic object, for example, a cluster, a group of clusters or a collapsing protocluster seen edgewise, it is likely to be marginal. Modern techniques permit a specific search for these lenses, which could constrain theories of structure formation in the Universe.

Clusters may be bright X-ray sources⁶, may cause inhomogeneity in the microwave background⁷, and are often elongated^{8,9}. For a lens that is elongated in projection, the minimal surface mass density necessary for splitting images is smaller^{1,4} by a factor ~ 2 than for round lenses^{3,10}. I shall hereafter discuss such lenses (which may be superclusters or clusters) and refer to them as 'pea-pods'. (Lensing by galaxies in clusters is not considered here; the lensing in question is that by clusters and superclusters taken as density distributions smoothed on the scale of an arc second.)

The following definitions and known facts are necessary for understanding the simple lens examples below.

Lensing of distant sources can be described as a single-valued mapping $\mathbf{r} \rightarrow \mathbf{s}$, where $\mathbf{r}$ is a two-component vector of angles

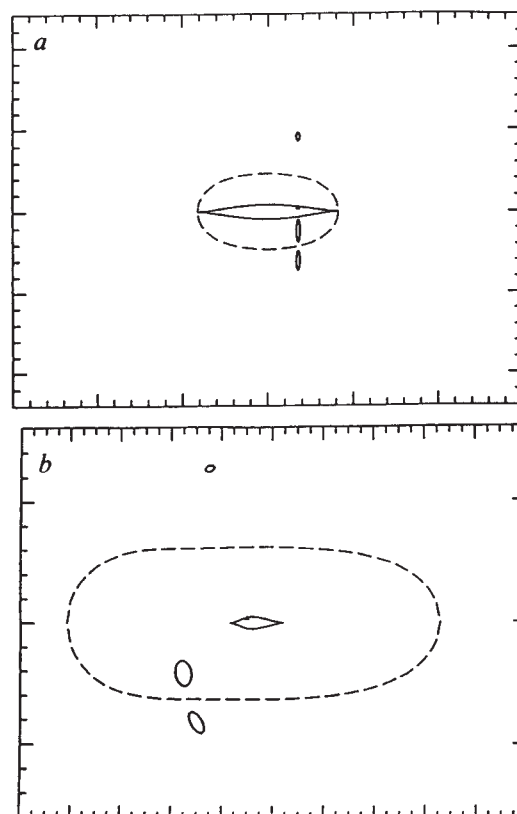


Fig. 1 Multiple imaging by marginal gravitational lenses. The figures are in projection on the sky on an arbitrary scale. Light solid lines are the source plane caustics inside which the source must reside to be multiply imaged. Broken lines are the image-plane caustics on which pairs of images merge and disappear. The triangle marks the source position. The heavy solid ellipses represent images; their area is proportional to the amplification and the shape is that of an infinitesimal circular source. The image separation is of the order of the size of the image-plane caustic. It is smaller than the lens vertical scale and larger than the source-plane caustic vertical scale. *a*, A very elongated, subcritical lens of elliptic potential (4) with $\kappa_0 = 0.65$ and $\epsilon = 0.9$. The vertical scale is stretched by a factor of 20 to show the details. *b*, A marginal lens made of two spheres of potential (4) with $\epsilon = 0$ each, different core radii and central convergences.

describing the image position in the sky, and $\mathbf{s}$ is a similar vector describing the 'unlensed' source position, i.e. the position at which it would be seen if the lens were absent. The inverse mapping can be multivalued, producing multiple images of the same source. A single-redshift stationary gravitational lens is described (similarly to formulations^{11,12} that exploit a modified Fermat principle) by a Lagrangian mapping^{13,14},

$$\mathbf{s} = \mathbf{r} - \nabla_{\mathbf{r}} \Phi(\mathbf{r}) \quad (1)$$

The effective potential is determined by a two-dimensional Poisson equation, $\nabla_{\mathbf{r}}^2 \Phi = 2\kappa(\mathbf{r})$. The line-of-sight convergence is $\kappa(\mathbf{r}) = \Sigma(d_{ol}\mathbf{r})/\Sigma_{cr}$, where the critical surface mass density is $\Sigma_{cr} = [(4\pi G/c^2)(d_{ol}d_{ls}/d_{os})]^{-1}$, and d_{ol} , d_{ls} and d_{os} are the observer-lens, lens-source and observer-source angular diameter distances.

The simplest parameter space necessary to describe a marginal lens is a three-dimensional source parameter space $(\mathbf{s}, \kappa_0)$, which is a direct product of the source plane $(\mathbf{s})$ and the one-dimensional space of the value of convergence, κ_0 , at a prominent point, say the maximum of the surface mass density (note that κ_0 depends on both z_l and z_s). In such a description, $\kappa(\mathbf{r}) = \kappa_0 K(\mathbf{r})$, where the function $K(\mathbf{r})$ determines a fixed lens configuration. The addition of a parameter to the image plane, $\mathbf{r}$, produces an image space, $(\mathbf{r}, \kappa_0)$.

* Present address: Physics Department, The Weizmann Institute, Rehovot 76100, Israel.

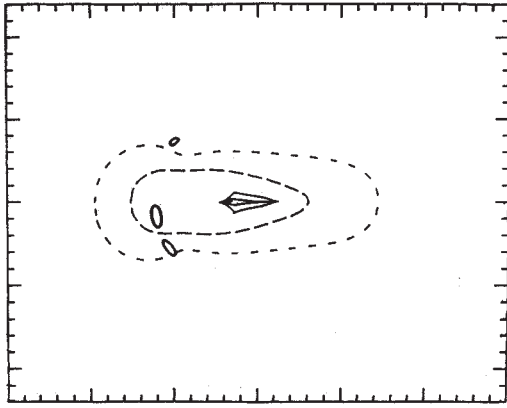


Fig. 2 Imaging of sources at $z_s = 1$ and $z_s = 2$ by the same lens, for the values of parameters given in the text. The lens is similar to that of Fig. 1b. The light solid lines are the source-plane caustics for $z_s = 1$ (the smaller area) and for $z_s = 2$. The long-dashed line is the image-plane caustic for sources at $z_s = 1$ at which the bright pairs of images merge. The heavy solid ellipses represent, as in Fig. 1, images of a particular source at $z_s = 1$. The short-dashed line is the image-plane caustic for sources at $z_s = 2$. Images of unsplit sources must lie outside this caustic, at a distance of order of half the width of the area inside (the borderline for unsplit images is not shown). It is possible to see unsplit images of a $z_s = 2$ source very close to the bright pair of images of a $z_s = 1$ source, and at the same time to see another group of split images of a $z_s = 1$ source farther away. Such an unperturbed lens cannot be a model of Q1146 + 111, as all the 3 images have large amplification. Superposed galaxies can affect the images, in particular the one inside the caustic can be split into several images¹³ which can possibly be fainter and missed in observation.

The regions in the source parameter space that produce different image multiplicities are separated by caustics, which are folds and higher singularities of the mapping. Images merge and disappear (appear) in pairs on the corresponding caustics in the image space. For a source inside caustics more than one value of r corresponds to one value of s .

The density bias, that is, the selection effect by which 'pea-pods' found responsible for multiple imaging tend to be marginal lenses, is in fact the bias to lensing near the 'bottom' of a three-dimensional lens caustic⁵, that is, in a narrow interval of values of κ_0 . The selection effect results from the observed cluster distribution in surface mass densities.

One of the eigenvalues of the amplification matrix, $\mathcal{A} = \partial r / \partial s$, changes sign on a caustic. (As this eigenvalue has a singularity on a caustic, it is easier to use the inverse matrix, $\mathcal{A}^{-1}$, for which an eigenvalue is zero on a caustic.) This property is a convenient tool to examine the ability of a given lens to split images.

A supercritical central surface mass density, that is, $\kappa_0 > 1$, is necessary for splitting images by a monotonic, spherical mass distribution¹⁰, whereas a lens with $\kappa(r) < \kappa_{\text{sup}}$ for all r can split images for any given $\kappa_{\text{sup}} > 0$, if the configuration is sufficiently aspherical, for instance, wedge-like⁴.

Distributions of galaxies with significant density contrasts and roughly wedge-like geometries have been observed in clusters of the Rood-Sastry types B and L¹⁵, in the redshift surveys¹⁶, strings of galaxies like that in Bootes¹⁷. Wedge-like cusp configurations arise naturally in the pancake^{9,18} and explosive (ref. 19; J. P. Ostriker and D. Weinberg, personal communication) theories of galaxy formation (merging shells in the latter).

The wedge-like configurations observed so far are all at small z_l , so that Σ_{cr} for them is much larger than it would be at an optimal redshift^{1,2}. It may in fact be that even at the optimal redshifts and orientations, the existing wedge-like mass concentrations cannot produce multiple images of large enough separation to allow identification of the wedge-like lens. For

instance, images separated by a fraction of an arc second could be attributed to a galaxy too faint to be seen.

Properties (1)–(3) make it possible to identify more ordinary and less subcritical marginal 'pea-pods'.

A marginal lens must be subcritical. Indeed, it follows from the definitions above that (i) $\kappa = 1 - \text{tr} \mathcal{A}^{-1}/2$; (ii) for a lens unable to split images, both eigenvalues of $\mathcal{A}$ are positive and $\kappa < 1$ for all r ; (iii) for a marginal lens, one eigenvalue is positive and another is small negative in a small region of the source plane, so that the lens is still subcritical, $\kappa < 1$, for all r .

To demonstrate how the density bias works consider a population of 'pea-pods' with the same $K(r)$ but κ_0 different from one 'pea-pod' to another (such a distribution can be produced, for instance, by distributing identical 'pea-pods' along the line of sight). Assume that the probability distribution in κ_0 declines steeply near $\kappa_0 \sim \kappa_{\text{min}}$ (and beyond, that is, for $\kappa_0 < \kappa_{\text{min}}$). Then the most probable maximal convergence or a 'pea-pod' found to split images is $\kappa_0 \sim \kappa_{\text{min}}$. A mixture of populations with different $K(r)$, for all of which $\kappa_{\text{min}} < 1$, results in a high probability for an observed case to be marginal and subcritical.

Properties (1)–(3) result from the following property of marginal lenses of arbitrary configurations (which are not too symmetrical or too special in the sense that all the coefficients of Taylor expansion of the effective potential are determined by order of magnitude by the angular scale of the lens, except for two coefficients which are 0 at the tip of the three-dimensional caustic⁵). Let $\kappa_0 = \kappa_{\text{min}} + \delta\kappa$, where $\delta\kappa \ll 1$. Let the angular scales of the mass distribution and the image separation be μ and δr respectively; δr is also the angular scale of the image-plane caustic. The source-plane caustic is always very elongated, resembling a sickle or lips^{5,18}, and can be assigned two angular scales, δs_1 and δs_2 . For a marginal lens⁵

$$\delta s_1 \sim \delta r \sim \mu \sqrt{\delta\kappa}, \quad \delta s_2 \sim \mu \delta\kappa^{3/2} \quad (2)$$

so that

$$\delta s_2 \ll \delta s_1 \sim \delta r \ll \mu \quad (3)$$

The probability for a random source's producing three images can be indicated by the area inside the source plane caustic, $A_s \sim \mu^2 \delta\kappa^2$.

A similar property is demonstrated by the following example, which is not arbitrary (elliptic symmetry and extreme elongation) but is taken for its simplicity. Consider a lens which can be approximated in its dense part by potential

$$\Phi(r) = \frac{1}{2} \kappa_0 a \sin^2 2\chi \sqrt{a^2 + \frac{x^2}{\cos^2 \chi} + \frac{y^2}{\sin^2 \chi}} \quad (4)$$

where $r = (x, y)$, and the equipotential contours are ellipses of eccentricity $e = \tan(\pi/4 - \chi)$. (Contours of equal surface mass density are not elliptical; however, $\kappa(r) > 0$ for all r . Elliptical potentials, although of limited applicability¹⁴, are simpler to handle than elliptical mass distributions²⁰.) Multiple imaging is possible (an eigenvalue of $\mathcal{A}$ is negative at the centre) for $\kappa_0 > \kappa_{\text{min}} = 1/[2 \max(\sin^2 \chi, \cos^2 \chi)]$. The lens is very elongated if, say, $\chi \ll 1$. The image-plane caustic ($\det \mathcal{A}^{-1} = 0$) is given by $x^2 + 3y^2/\chi^2 = 4a^2 \delta\kappa$. Mapping (1) yields the source-plane caustic. The y -scales of the image plane and source plane caustics are $a\chi\sqrt{\delta\kappa}$ and $a\chi\delta\kappa^{3/2}$; the x -scales are both $a\sqrt{\delta\kappa}$. The small parameter χ introduces additional scales; however, relations similar to (2) and (3) are obtained, and $A_s = (4\pi/\sqrt{3})a^2\chi\delta\kappa^2$. An example of such a lens is shown in Fig. 1a.

Figure 1b shows an example of a less special marginal lens (in the sense that it has only one line of symmetry and is not very elongated).

Properties (1) and (2) follow from the fact that the scale of the mass distribution may be traced by enhancement of galaxies, by microwave inhomogeneities^{7,21,22}, by a bright X-ray source⁶ or by a 'quasar cluster' produced by some amplification of quasars in a large part of the area of the 'pea-pod'.

Property (3) is demonstrated by the example in Fig. 2, which

corresponds to an $\Omega = 1$, Friedmann zero-pressure universe, empty-beam angular diameter distances (corresponding to all the mass of the Universe concentrated in clumps outside the light beam^{14,34}), $H_0 = 60 \text{ km s}^{-1}$, and a lens at redshift $z_l = 0.3$ (near the minimum of Σ_{cr} for source redshift $z_s = 1$). The dependence $A_s(z_l, z_s)$ is determined by d_{os} and Σ_{cr} . For the example of Fig. 2, for a source at $z_s = 1$ $d_{\text{os}} = 1,646 \text{ Mpc}$ and $\Sigma_{\text{cr}} = 0.69 \text{ g cm}^{-2}$. For a source at $z_s = 2$, the values of d_{os} , Σ_{cr} and A_s are modified by factors 1.14, 0.86 and 3.3, respectively.

To estimate the cluster surface mass densities consider an example of a rich and compact, approximately spherical and isothermal cluster with a line-of-sight velocity dispersion $\sigma_{\parallel} \sim 1500 \text{ km s}^{-1}$, core radius 0.3 Mpc and redshift $z_l = 0.4$; a source at $z_s = 2$, and the universe as that considered in the example above. Then $\Sigma_{\text{cr}} = 0.57 \text{ g cm}^{-2}$ and the central convergence is $\kappa_0 \sim 0.7$.

The properties of marginal lenses can explain some of the known observations and also they can allow us to search for this specific kind of lensing.

The dominance of subcritical lensing for superclusters may explain the failure to find alignment of extended radio sources^{23,24}. The area A_s may be $\sim \text{arc s}^2$, whereas the image separations are of the order of many arc seconds. Under such circumstances there cannot be any alignment on the scale of arc degrees sought by refs 23 and 24. Faint radio sources for which a shape can be resolved by modern techniques are separated by more than $10'$ on average²⁵. Such a source must fall within the caustic to be multiply imaged. Therefore, even if sampling on the scale of arc minutes were attempted, the statistical effect on alignment would be very small.

The most promising means to seek gravitational lensing by superclusters seems at present to be looking for multiply imaged objects in the fields of 'quasar clusters'.

Recently, a gravitational lens candidate pair of quasar images, Q1146+111 B, C, of very large separation was reported²⁶ on the basis of striking similarities of their spectra. Later, differences in emission lines were found^{27,35}, though it could be argued that the differences could be due to arrival time differences (which are $\sim 10^3$ years and can allow for variations in a volume of linear size $\sim 1 \text{ kpc}$). The probability of a chance coincidence must be smaller than that calculated by ref. 28, because they did not take into account the known and unusual spectral similarities. In any case the pair Q1146+111 B,C requires further investigation before it can be determined whether they are separate quasars or two images of the same quasar.

A way to distinguish between these possibilities (suggested by B. Paczyński) is to look at galaxies at the redshift of the quasar pair in the immediate area of the pair. If the pair is produced by marginal lensing, some of the galaxies may be unusually large for their redshift, whereas some may be actually crossed by the lens caustics and have unusual lopsided shapes⁵.

Regardless of the fate of Q1146+111 B,C, this pair may be either a representative or a prototype of groups of quasar images with similar properties still to be discovered. Taking it as the latter, it is interesting to note that the pair exhibits the properties of marginal lensing: there is no prominent concentration of galaxies in the area of the pair, there is the 'quasar cluster'²⁹, there are unsplit images of quasars with larger redshifts close to the pair on the scale of image separation, and the microwave inhomogeneity was not detected on the angular scale of image separation²² though it should be detectable for standard-type lens models^{7,22,30,31}.

There are a few known 'quasar clusters'²⁹ and groups of quasar images of close redshift and separations of the order of arc minutes³². Some of them may be the result of gravitational lensing by superclusters. An investigation of these areas of the sky by currently available techniques (for example, ref. 33 and J. E. Gunn, personal communication) could reveal multiple images of galaxies as well as quasars, which could yield information on supercluster structures and masses.

I thank Y. Avni, J. H. van Gorkom, J. R. Gott, J. E. Gunn, A. J. S. Hamilton, A. Kashlinski, C. G. Lacey, T. R. Lauer, M. Milgrom, J. P. Ostriker, B. Paczyński, H. J. Rood, E. L. Turner and D. Weinberg for helpful advice, comments, conversations, discussions and remarks. The work was supported by the Dr Chaim Weizmann postdoctoral fellowship of the Princeton University and by Stiftung Volkswagenwerk at the Weizmann Institute.

Received 7 July; accepted 30 October 1986.

- Sanders, R. H., van Albada, T. S. & Oosterloo, T. O. *Astrophys. J.* **278**, L91-L94 (1984).
- Narayan, R., Blandford, R. & Nityananda, R. *Nature* **310**, 112-115 (1984).
- Turner, E. L., Ostriker, J. R. & Gott, J. R. *Astrophys. J.* **284**, 1-22 (1984).
- Subramanian, K. & Cowling, S. A. *Mon. Not. R. Astr. Soc.* **219**, 333-346 (1986).
- Kovner, I. *Astrophys. J.* (in the press).
- Sarazin, C. L. *Rev. mod. Phys.* **58**, 1-115 (1986).
- Ostriker, J. P. & Vishniac, E. T. *Nature* **322**, 804 (1986).
- Oort, J. H. A. *Rev. Astr. Astrophys.* **21**, 373-428 (1983).
- Efstathiou, G. & Silk, J. *Fundam. Cosmic Phys.* **9**, 1-138 (1983).
- Jaroszyński, M. & Paczyński, B. in *Proc. Second Eur. Reg. Mg (IAU) (Memorie Soc. astr. ital.* **45**) 673-480 (1974).
- Schneider, P. *Astr. Astrophys.* **143**, 413-420 (1984).
- Blandford, R. & Narayan, R. *Astrophys. J.* **310**, 568-582 (1986).
- Kovner, I. *Astrophys. J.* (in the press).
- Kovner, I. *Astrophys. J.* (in the press).
- Struble, M. F. & Rood, H. J. *Astr. J.* **87**, 7-46 (1982).
- de Lapparent, V., Geller, M. J. & Huchra, J. P. *Astrophys. J.* **302**, L1-L5 (1986).
- Tago, E., Einasto, J. & Saar, E. *Mon. Not. R. Astr. Soc.* **218**, 177-184 (1986).
- Arnold, V. I., Shandarin, S. F. & Zel'dovich, Ya. B. *Geophys. Astrophys. Fluid Dyn.* **20**, 111-130 (1982).
- Vishniac, E. T., Ostriker, J. P. & Bertschinger, E. *Astrophys. J.* **291**, 399-416 (1985).
- Bourassa, R. R., Kantowski, R. & Norton, T. D. *Astrophys. J.* **185**, 747-756 (1973).
- Kaiser, N. & Stebbins, A. *Nature* **310**, 391-393 (1984).
- Stark, A. A., Dragovan, M., Wilson, R. W. & Gott, J. R. *Nature* **322**, 805 (1986).
- Sanders, R. H. *Nature* **309**, 35-37 (1984).
- Kapahi, V. K., Subramanian, R. & Singal, A. K. *Nature* **313**, 463-465 (1984).
- Windhorst, R. thesis, Univ. Leiden (1984).
- Turner, E. L. *et al.* *Nature* **321**, 142-144 (1986).
- Shaver, P. A. & Cristiani, S. *Nature* **321**, 585-586 (1986).
- Phinney, E. S. & Blandford, R. D. *Nature* **321**, 570-571 (1986).
- Arp, H. & Hazard, C. *Astrophys. J.* **240**, 726-736 (1980).
- Paczynski, B. *Nature* **321**, 419-420 (1986).
- Gott, J. R. *Nature* **321**, 420-421 (1986).
- Paczynski, B. *Nature* **319**, 567-568 (1986).
- Tyson, J. A., Valdes, F., Jarvis, J. F. & Mills, A. P. *Astrophys. J.* **281**, L59-L62 (1984).
- Dyer, C. C. & Roeder, R. C. *Astrophys. J.* **174**, L115-L117 (1972).
- Huchra, J. P. *Nature* **323**, 784-786 (1986).

Morphology of the geomagnetic field and implications for the geodynamo

David Gubbins*† & Jeremy Bloxham†

* Bullard Laboratories, Cambridge University, Cambridge CB3 0EZ, UK

† Department of Earth and Planetary Sciences, Harvard University, Cambridge, Massachusetts 02138, USA

Maps of the radial component of the magnetic field at the surface of Earth's core for 1715-1980 show, on average, a pattern of four lobes, placed symmetrically about the Equator, and zero flux near the poles. Time variations are largely confined to the Atlantic hemisphere (90° E to 90° W). We propose this average field to be the true dynamo-generated field; that equatorial symmetry is a fundamental consequence of the dynamo equations; and that the polar patches are because of the dynamical influence of the inner core. The main lobes are 120° apart and we propose that the flux from a missing third pair of lobes, which should appear symmetrically near the Greenwich meridian, has been re-distributed by near-surface fluid flow to generate secular variation in the Atlantic hemisphere.

New methods, based on inverse theory, of analysing magnetic measurements^{1,2} have yielded maps of the magnetic field at the

† Present address: Research School of Earth Sciences, Australian National University, Canberra, ACT 2601, Australia.

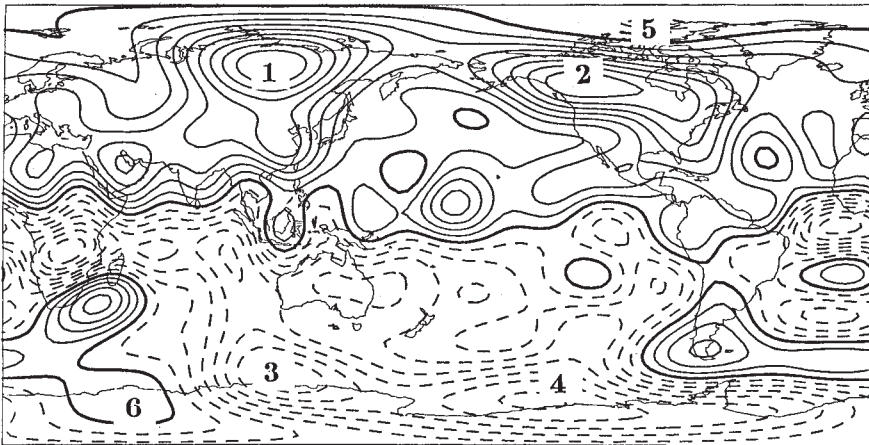


Fig. 1 Map of the radial component of the magnetic field at the core-mantle boundary for 1980 (ref. 3). Contour interval is 100 μT ; solid contours represent flux into the core, broken contours flux out of the core; bold contours represent zero radial field. The two main pairs of lobes (1,3) and (2,4) are indicated, as are the patches of low radial field (5 and 6) near the poles.

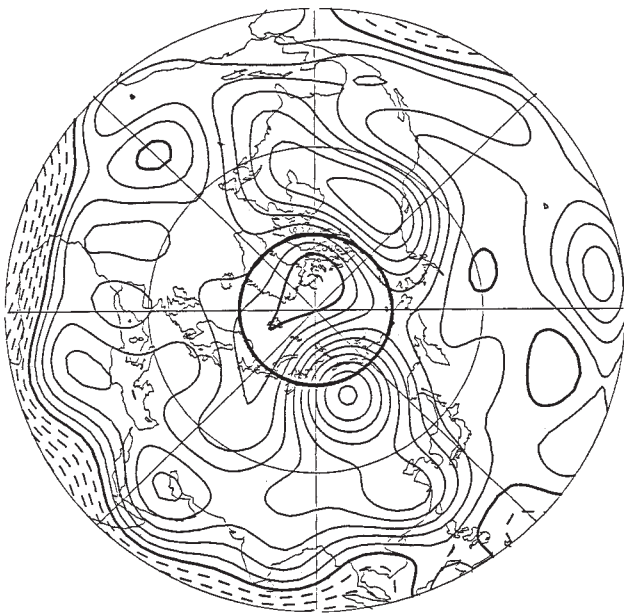


Fig. 2 As Fig. 1 but viewed from the North Pole in a Lambert azimuthal equal-area projection. The projection of the inner core onto the core-mantle boundary is described by the bold circle centred on the North Pole.

core-mantle boundary (CMB) with greatly improved resolution and accuracy³⁻⁵. The fields are displayed by their radial component; a map for 1980, based on data from the Magsat satellite, assuming an insulating mantle, is shown in Fig. 1. The earliest map draws on data from AD 1695^{5,6} (ref. 5 and J.B. and D.G., in preparation). We can track movement of radial features by comparing maps at different epochs. Drifting features, which almost invariably move westwards, have been confined to the Atlantic hemisphere for the entire period—long enough to show features forming and disappearing at the edges of the region. The observation of low secular variation at the CMB beneath the Pacific should be distinguished from the well-known observations of low secular variation in this region at the Earth's surface based on palaeomagnetic measurements⁶.

Some features are stationary. By far the most striking of these are the four concentrations of flux (lobes) marked 1-4 in Fig. 1. With a typical westward drift rate of $0.2^\circ \text{ yr}^{-1}$ we would expect these features to have drifted by over 50° in longitude during this period: such a large drift would be clearly detectable and has most certainly not occurred. The lobe beneath Canada appeared to split in the nineteenth century, possibly owing to

an interaction with a moving feature (J.B. and D.G., in preparation). Low polar flux is another persistent feature (marked 5, 6 in Fig. 1). The North-Pole patch is consistent between epochs, the southern one less so—it is displaced some distance from the pole in 1980, perhaps because the patch of negative flux beneath South America has displaced it and lobe 3 (J.B. and D.G., in preparation).

We ignore all drifting features and the smaller static ones, like that in the central Pacific: there remains an average field (AF) consisting of four main lobes and zero flux near the poles. At the Earth's surface it is predominantly dipolar, but otherwise quite dissimilar from the present-day field. It has two remarkable properties: antisymmetry about the Equator (pairs of lobes lie on the same longitude, near 120° E and 120° W), and zero intensity near the poles (where a dipole field would achieve maxima). Equatorial antisymmetry requires that $\{g_l^m, h_l^m\} = 0$ if $l-m$ is even, in terms of standard geomagnetic coefficients⁷.

We postulate that AF is the main standing field generated by the dynamo. The SV may partake in the generation process (as in a MAC-wave dynamo⁸, for example), or it may simply be a consequence of near surface motions unrelated to dynamo action.

Dynamo-generated fields allowed the observed hemispheric symmetry. Solutions to the equations governing incompressible flow driven by buoyancy (thermal or compositional), Coriolis, and magnetic forces may satisfy^{9,10}

$$P(\pi - \theta) = -P(\theta)$$

$$T(\pi - \theta) = T(\theta)$$

$$p(\pi - \theta) = p(\theta)$$

$$t(\pi - \theta) = -t(\theta)$$

$$S(\pi - \theta) = S(\theta)$$

where θ is a colatitude, S is temperature (or composition), u velocity, and $\mathbf{B}$ magnetic field with

$$\mathbf{u} = \nabla \wedge t\hat{\mathbf{r}} + \nabla \wedge \nabla \wedge p\hat{\mathbf{r}}$$

$$\mathbf{B} = \nabla \wedge T\hat{\mathbf{r}} + \nabla \wedge \nabla \wedge P\hat{\mathbf{r}}$$

The opposite symmetry is not allowed. Hemispheric symmetry has been used in spherical convection and dynamo calculations to speed computation^{9,10}. The hemispheric solution is believed more unstable, and therefore more physically realisable.

The dynamics of the core are dominated by Coriolis forces, the boundaries, and possibly magnetic forces associated with large toroidal field. In the regions above and below the inner core the boundaries are roughly perpendicular to the rotation axis: these regions resemble plane layers. In equatorial regions the boundaries are roughly parallel to the rotation axis, resembling the geometry of a cylindrical annulus rotating about its axis. Both these geometries have been studied extensively and

exhibit quite different dynamical behaviour¹¹; accordingly, we expect the behaviour inside the projection of the inner core, along the rotation axis, onto the CMB, which is a circle of 20.4° (Fig. 2), to be different from that outside. The positions of the two main lobes in each hemisphere are consistent with convection rolls parallel to the rotation axis and tangential to the inner core. In Busse's simple model¹² each convection roll has flow along its length induced by the boundary providing the helicity required for dynamo action. Downwelling flow along the rolls will concentrate flux as observed; alternating with these rolls we expect other rolls along which the flow is towards the CMB. Such a roll should be centred on 180° (midway between the concentrations of flux at 120° E and 120° W), where we observe low flux as a result of the upwelling.

The location of the main lobes near 120° W and 120° E suggests a missing third pair of lobes near Greenwich meridian resulting in a three-fold azimuthal symmetry. This would result from a total of six convection rolls, three with flow away from the CMB, and three with flow towards the CMB. The 'missing' third pair of lobes would be in the Atlantic Ocean where the secular variation is strong. We propose that the vigorous fluid motions associated with this stronger secular variation have prevented their formation.

The inner core circle is comparable in size with the zero flux

patch observed there (Fig. 2); this may be a result of the different dynamics in this region.

We have presented a highly idealized model of the geodynamo, for a spherical symmetric Earth, with a three-fold azimuthal symmetry and perfect antisymmetry about the equator. In a companion paper we argue that this simple picture is complicated by the effect of lateral heterogeneities in the lower mantle on the flow in the core, which may explain the asymmetrical nature of the secular variation and control the azimuthal orientation of the rolls and other features.

This work was supported by NERC grant GR3-3475 and NSF grants EAR-8317594 and EAR-8608890.

Received 9 July; accepted 13 November 1986.

1. Shure, L., Parker, R. L. & Backus, G. *Phys. Earth planet. Inter.* **28**, 215-229 (1982).
2. Gubbins, D. *Geophys. J. R. astr. Soc.* **73**, 641-652 (1983).
3. Gubbins, D. & Bloxham, J. *Geophys. J. R. astr. Soc.* **80**, 695-713 (1985).
4. Shure, L., Parker, R. L. & Langel, R. A. *J. geophys. Res.* **90**, 11505-11512 (1985).
5. Bloxham, J. & Gubbins, D. *Nature* **317**, 777-781 (1985).
6. Doell, R. R. & Cox, A. *Science* **171**, 248-254 (1971).
7. Chapman, S. & Bartels, J. *Geomagnetism* (Oxford University Press, 1962).
8. Braginsky, S. I. *Sov. Phys. JETP* **20**, 1462-1471 (1965).
9. Young, R. E. *J. Fluid Mech.* **63**, 695-721 (1974).
10. Gubbins, D. *Geophys. J. R. astr. Soc.* **42**, 295-305 (1975).
11. Chandrasekhar, S. *Hydrodynamic and Hydromagnetic Stability* (Oxford University Press, 1961).
12. Busse, F. H. *Geophys. J. R. astr. Soc.* **42**, 437-460 (1975).

Thermal core-mantle interactions

Jeremy Bloxham* & David Gubbins†‡

* Department of Earth and Planetary Sciences, Harvard University, Cambridge, Massachusetts 02138, USA

† Bullard Laboratories, Cambridge University, Cambridge CB3 0EZ, UK

Maps of the magnetic field at the core-mantle boundary for 1715-1980 reveal static features in the field and an absence of westward drift from much of the core-mantle boundary. The static features suggest that flow in the core is coupled to the mantle. We examine different coupling mechanisms and predict lateral inhomogeneity at the core-mantle boundary. Comparison with models of the lower mantle and core-mantle boundary derived seismically and from the geoid is encouraging, though their resolution is currently inadequate for a full comparison to be made with the much better-resolved magnetic field.

In an earlier paper¹ we presented models of the magnetic field at the core-mantle boundary (CMB) at approximately 60 yr intervals from 1715 to 1980. We showed that the secular variation (SV) is confined mainly to the hemisphere 90° E to 90° W, where its character has remained unaltered throughout the 265 yr time-span of the models. Elsewhere we identified static features in the field at the CMB. We suggested that this confinement of SV to one hemisphere and the presence of many static features is the result of coupling of flow in the core to the mantle. Mean zonal flows are expected on theoretical grounds^{2,3}; the absence of westward drift of features in the field at the CMB in the Pacific hemisphere suggests that flow in the core may be coupled to the mantle. The flux patches beneath Siberia and Canada, which we have identified with convection rolls⁴, should also drift⁵: no drift of these features is observed.

Stationary patches of intense field (static flux bundles) are labelled 1-5 in Fig. 1; they were attributed to flux concentration by downwelling fluid, associated with cold mantle, balancing diffusive decay¹. Likewise, upwelling may form zero flux patches (labelled 6-9 in Fig. 1) by sweeping field lines away, although we believe the polar patches (6 and 9) to be associated with the

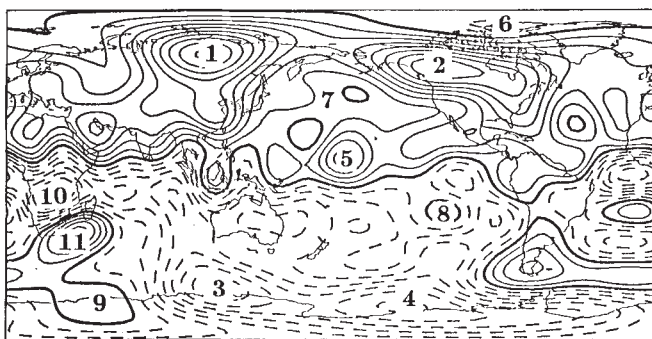


Fig. 1 Map of the radial component of the magnetic field at the core-mantle boundary for 1980 (ref. 6), produced assuming an insulating mantle. Contour interval is 100 μ T; solid contours represent flux into the core, broken contours flux out of the core; bold contours represent zero radial field. The following features are indicated: static flux bundles (1-5); static zero flux patches (6-9); core spots (10-11).

dynamical influence of the inner core⁴. In regions where the subsurface toroidal field is strong, upwelling will concentrate intense field beneath the CMB^{7,8} leading to the formation of core spots. The core spot pair (10-11) appeared to form under the western Indian Ocean and intensified beneath southern Africa.

We propose that flow in the core is thermally coupled to the lower mantle, with upwelling beneath hot regions of the lower mantle and downwelling beneath cold regions. Such an argument may be too simplistic: the problem of coupled convection between two fluids of such vastly different properties requires careful analysis. Jones⁹ has argued that lateral temperature variations on the CMB will only affect the flow in a thin layer near the CMB. If the upper regions of the core are strongly stably stratified then such effects will be confined to a thin layer, but if we assume the whole core convects then lateral temperature variations in the lowermost mantle may affect a larger region. Free convection in the core, resulting most probably from crystallization at the inner core boundary, drives the dynamo. Free convection is driven in the lower mantle, in large part, by the heat flux from the core. Convection in the lower mantle results in large lateral temperature variations just above

‡ Present address: Research School of Earth Sciences, Australian National University, Canberra, ACT 2601, Australia.

the CMB: these lateral temperature variations act back on the core to force convection in the core. The forced convection in the core must be relatively weak otherwise the heat flux from beneath hot regions of the mantle to beneath cold regions will be sufficient to remove any lateral temperature variations from the lower mantle on a very short timescale. However, even if the forcing is very weak compared with the free convection it may still be sufficient to affect the pattern of convection in the core, by forcing alignment of convection cells in the core with thermal anomalies in the lowermost mantle.

We can make this argument more quantitative. Lateral temperature variations forcing core flow may be roughly estimated by balancing Coriolis and buoyancy torques:

$$\Omega U \approx \alpha g \Delta T \quad (1)$$

where $\alpha = 5 \times 10^{-6}$ is the coefficient of thermal expansion¹⁰, g the acceleration due to gravity, Ω the diurnal rotation rate and T the temperature. Taking $5 \times 10^{-4} \text{ m s}^{-1}$ as a typical value of U , then the lateral temperature variations on the CMB $\Delta T \approx 5 \times 10^{-4} \text{ K}$. From the point of view of mantle convection the core fluid acts to make the CMB isothermal: but the heat flux through the CMB will vary laterally.

Consider hot and cold regions a distance L apart. Core fluid flows from hot to cold in time L/U , cooling by ΔT to a depth $(\kappa L/U)^{1/2}$, the thermal diffusion length, where $\kappa = 5 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ is the thermal diffusivity¹¹. The heat flux, H , carried by this mass of fluid is:

$$H = \rho C_p (\kappa L/U)^{1/2} [\Delta T / (L/U)] \quad (2)$$

This is the lateral variability in the heat flux necessary to force convection in the core. Taking the density $\rho = 10^4 \text{ kg m}^{-3}$ (ref. 11), the specific heat capacity $C_p = 700 \text{ J kg}^{-1} \text{ K}^{-1}$ (ref. 13) and $L = 1,000 \text{ km}$, we obtain $H \approx 0.1 \text{ mW m}^{-2}$, which is very reasonable compared with the total heat flux from the core of the order of 10 mW m^{-2} (ref. 11), implying lateral variability in heat flux of 1%.

We can use this result to calculate the size of the lateral temperature variations which must be maintained in the lower mantle, above the thermal boundary layer at the CMB, to force this flow. Assuming a thickness of 100 km for the thermal boundary layer¹², and a thermal conductivity of $10 \text{ W m}^{-1} \text{ K}^{-1}$ (ref. 12), we find that lateral temperature variations of $\sim 1 \text{ K}$ are sufficient. These calculations suggest that forcing of flow in the core by lateral temperature variations in the lower mantle will almost certainly be a significant effect.

We have assumed a molecular value for the thermal diffusivity. The presence of turbulence in the thermal boundary layer would increase the effective diffusivity. The usual expectation is that turbulence acts to increase diffusivities so as to make the Prandtl numbers unity. The molecular magnetic Prandtl number is 0.1 (ref. 11); because the magnetic diffusivity is unlikely to increase greatly, the expected increase in the effective thermal diffusivity is then only a factor of 10 (the increase in viscosity would be very much larger) which is not large enough to vitiate the conclusions of our calculation.

Topography of an isothermal CMB will also affect convection because isotherms no longer coincide with equipotentials. Tilting the boundary is analogous to tilting g in plane-layer convection, which is known to set up large convection cells¹³. The sense is downward for a depression into the fluid, the same as for a cold region. The flow is determined by the aspect ratio of the bump and the vertical superadiabatic temperature gradient in the core. With a high superadiabatic temperature gradient of 1 K km^{-1} in the thermal boundary layer at the top of the core the equivalent ΔT is 10^{-3} K , for a bump of height 1 km and width $1,000 \text{ km}$, which is large enough to influence convection, but such a bump would be warmed by the core flow in a few hundred years, so that the thermal effect of a bump appears slight. In any case this thermal effect of a bump, as distinct from the topographic, or mechanical, effect that we consider below,

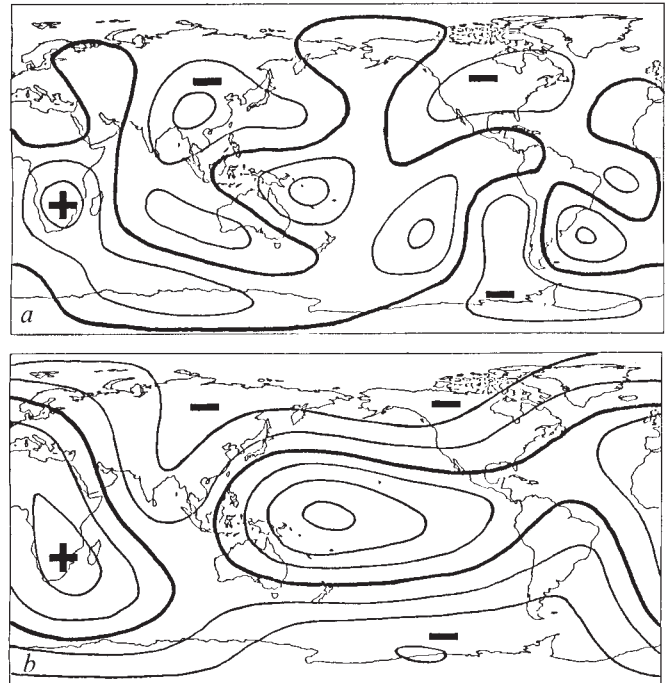


Fig. 2 *a*, Map of lateral variations in seismic P -wave velocity at the CMB after Dziewonski¹⁴. The contour interval is 0.5%. Maximum spherical harmonic degree, six. *b*, Map of the CMB after Hager *et al.*¹⁵. The contour interval is 500 m. Maximum spherical harmonic degree, three. In each map the + sign represents a region of anomalously hot mantle (elevated CMB) corresponding to the core spots beneath southern Africa (upwelling flow), and the - signs represent regions of anomalously cold mantle corresponding to static flux bundles (downwelling flow). Although there is certainly an encouraging indication that regions of upwelling in the core are associated with regions of hot mantle, and downwelling with cold mantle, the resolution is insufficient for a full comparison to be made.

is of the same sign as the effect of thermal anomalies in the lower mantle, because depressions of the CMB into the core are believed to correspond to regions of anomalously cold mantle.

Mantle temperature inhomogeneities can be mapped from variations in seismic velocity, geoid anomalies and laws relating them to ρ and T (refs 14 and 15). Cold mantle will have high seismic velocity and correspond to depressed CMB; hot mantle the reverse. In Fig. 2 we compare these magnetically inferred lateral homogeneities at the CMB with a map of lateral variations in seismic P -wave velocity in the lowermost mantle¹⁴, and with a map of topography of the CMB¹⁵. Although the correlation is certainly encouraging, and lends support to our hypothesis of thermal coupling, the resolution of these maps is insufficient for a full comparison to be made with the magnetic field map in Fig. 1 that contains information out to spherical harmonic degree 12.

We should consider other mechanisms besides thermal effects that may result in a coupling of flow in the core to the mantle and explain the correlation with lower mantle inhomogeneities: we consider the mechanical effect of bumps, mentioned above, and electromagnetic coupling. The terms topographic core-mantle coupling and electromagnetic core-mantle coupling are normally applied to the exchange of angular momentum between the core and the mantle in studies of decade changes in the length of day¹⁶. Our concern here is rather different: we seek mechanisms by which the pattern of convection in the core may be fixed by inhomogeneities in the lower mantle.

Bumps on the CMB may also have a mechanical effect on the flow in the core, but it seems improbable that undulations a few kilometres in height but several thousand kilometres in wavelength will have a very great effect on core motions, a view

supported by Anufriyev and Braginsky¹⁷. Certainly regions of pronounced upwelling and downwelling are unlikely to result; entrainment of Taylor columns by bumps is a possibility, but this cannot explain upwelling beneath regions of elevated CMB. Hide and Malin¹⁸ correlated the long-wavelength geoid with the rotated non-dipole field, which is quite different from the correlation we are proposing. It is very hard to explain why the field pattern should rotate while retaining a signature of the CMB; in fact, the significance of Hide and Malin's correlation itself is in some doubt¹⁹.

Flow in the highly electrically conducting core is electromagnetically coupled to the very much poorer conducting lower mantle: the movement of magnetic field lines in the mantle, as a result of advection due to fluid motions in the core, induces an electromotive force in the lower mantle. Although this probably has an important effect on the relative rotation of the core and mantle¹⁶, and the effect is likely to vary laterally due to the temperature dependence of mantle conductivity, it is difficult to see why upwelling flow would be concentrated beneath regions of hot mantle and downwelling beneath cold regions as a result of variations in electromagnetic coupling.

Chemical inhomogeneity in the lower mantle may produce seismic velocity and density variations unrelated to the mantle temperature and destroy the concurrence of cold regions with fast, dense mantle, although chemical and thermal variations may be related. The favourable comparison with the magnetic field suggests a predominantly thermal origin for the large scale variations in seismic velocity observed in the lower mantle.

Determination of lateral variations in seismic velocity in the lower mantle is difficult; determination of CMB topography from the geoid and mantle density variations is also very uncertain as it involves a host of untested assumptions and unknown

parameters (such as the viscosity profile of the mantle). Now, such models do not approach the accuracy with which we know the magnetic field at the CMB, for which terms up to at least spherical harmonic degree ten are well determined throughout the twentieth century, and terms up to degree eight back to the early eighteenth century. New initiatives aimed at the collection of higher quality global seismic data should improve the resolution of lateral variations in the lower mantle: it will be interesting to see if such improvements reveal some of the smaller thermal anomalies (such as that associated with the feature beneath the central Pacific labelled 5 in Fig. 1) which we believe to exist.

This work was supported by NSF grants EAR-8317594 and EAR-8608890 and by NERC grant GR3-3475. We thank Greg Houseman for useful discussion.

Received 25 July; accepted 13 November 1986.

1. Bloxham, J. & Gubbins, D. *Nature* **317**, 777-781 (1985).
2. Taylor, J. B. *Proc. R. Soc., Lond. A* **274**, 274-283 (1963).
3. Busse, F. H. & Hood, L. L. *Geophys. Astrophys. Fluid Dynam.* **21**, 59-74 (1982).
4. Gubbins, D. & Bloxham, J. *Nature* **325**, 509-511 (1987).
5. Busse, F. H. *J. Fluid Mech.* **44**, 441-460 (1970).
6. Gubbins, D. & Bloxham, J. *Geophys. J. R. astr. Soc.* **80**, 695-713 (1985).
7. Allan, D. W. & Bullard, E. C. *Rev. mod. Phys.* **30**, 1087-1088 (1958).
8. Bloxham, J. *Geophys. J. R. astr. Soc.* **87**, 669-678 (1986).
9. Jones, G. M. *J. geophys. Res.* **82**, 1703-1709 (1977).
10. Gubbins, D. *Rev. Geophys. Space Phys.* **12**, 137-154 (1974).
11. Gubbins, D., Masters, T. G. & Jacobs, J. A. *Geophys. J. R. astr. Soc.* **59**, 57-99 (1979).
12. Jeanloz, R. & Richter, F. M. *J. geophys. Res.* **84**, 5497-5504 (1979).
13. Weber, J. E. *Int. J. Heat Mass Transfer* **16**, 961-970 (1973).
14. Dziewonski, A. M. *J. geophys. Res.* **89**, 5929-5952 (1984).
15. Hager, B. H., Clayton, R. W., Richards, M. A., Comer, R. P. & Dziewonski, A. M. *Nature* **313**, 541-545 (1985).
16. Courtillot, V. & Le Mouél, J.-L. *Nature* **311**, 709-716 (1984).
17. Anufriyev, A. P. & Braginsky, S. I. *Geomagn. Aeron.* **17**, 492-496 (1977).
18. Hide, R. & Malin, S. R. C. *Nature* **255**, 606-609 (1970).
19. Eckhardt, D. H. *Math. Geol.* **16**, 155-171 (1984).

Crustal thinning on the Aquitaine shelf, Bay of Biscay, from deep seismic data

B. Pinet*, L. Montadert*, R. Curnelle†, M. Cazes‡‡, F. Marillier‡, J. Rolet‡, A. Tomassino‡, A. Galdeano§, Ph. Patriat§, M. F. Brunet||, J. L. Olivet¶, M. Schaming#, J. P. Lefort**, A. Arrieta†† & C. Riaza††

* Institut Français du Pétrole, BP 311, 92506 Rueil Malmaison Cedex, France

† Société Nationale Elf-Aquitaine, Mission France, 31360 Saint Martory, France

‡ Université de Bretagne Occidentale, 6 Avenue le Gorgeu, 29283 Brest Cedex, France

§ Institut de Physique du Globe de Paris, 4 Place Jussieu, 75230 Paris Cedex 05, France

|| Groupe d'Etude de la Marge Continentale, Département de Géologie Dynamique, Université Pierre et Marie Curie, 4 Place Jussieu, 75230 Paris Cedex 05, France

¶ IFREMER, Centre de Brest, BP 337, 29273 Brest Cedex, France

Institut de Physique du Globe de Strasbourg, 5 rue Descartes, 67084 Strasbourg Cedex, France

** Centre Armorican d'Etude Structurale des Socles, Institut de Géologie, Campus de Beaulieu, 35042 Rennes Cedex, France

†† Hispanoil, Pez Volador 2, 28007 Madrid, Spain

‡‡ Société Nationale Elf-Aquitaine (Production), Direction Exploration, Tour Elf, Cedex 45, 92078 Paris la Défense, France

In recent years an increasing amount of new scientific data on the structure and nature of the whole continental crust has been acquired from deep seismic profiling. The Bay of Biscay profile was recorded in 1984 at sea along the Aquitaine platform for the French ECORS group in association with Hispanoil. It collected a unique set of two-ship multi-channel seismic data with constant offset and expanded spread profiles in a complex geological area. The data are of very good quality and contain clear Moho and

lower crust reflections as seen under most parts of Western Europe where similar experiments have been conducted¹⁻⁴. This profile provided for the first time the most convincing evidence for an anomalously thin crust below a rifted basin, the Parentis Basin, which is directly connected to the oceanic area of the Bay of Biscay. It gives new constraints for understanding the formation of extensional sedimentary basins.

The experiment was carried out along a 300-km-long line oriented north-south from the Armorican Shelf to the Cantabria margin (Fig. 1). A normal incidence seismic profile was shot and recorded up to 15 s by the *GECO Gamma* vessel using an 8,536 in³ air gun source at 2,000 psi and a 3-km-long streamer. At the same time shots were recorded up to 16 s by the IFP vessel *Résolution* steaming behind with a 7.5-km constant offset and towing a 2.4-km-long streamer to simulate a long-array spread. Processing was done by Elf-Aquitaine, providing a 30-fold CDP (common depth-point) stacked section for the near offset profile which was then migrated, and a 24-fold CDP stacked section for the large offset profile which was muted from 0 to 7.5 s to avoid refraction arrivals. A spectacular increase in energy from the deep reflectors as well as a remarkable improvement in the definition of the lower crust and of the Moho were obtained with the wide-aperture data which also helped in cancelling the diffractions (Fig. 2). At intervals along this profile, six expanding-spread profiles were also acquired. This latter survey showed that the main changes in acoustic facies or in the pattern of reflections observed on the vertical reflection data are associated with velocity discontinuities. Beneath the sediments which correspond to velocities of less than 5.7 km s⁻¹, the upper crust is characterized by velocities ranging from 5.9 to 6.2 km s⁻¹, while they range from 6.5 to 7.1 km s⁻¹ in the lower crust⁵. The two-way time corresponding to the location of the Moho is marked by a strong wide-angle reflection on each expanded spread profile (ESP) and an abrupt disappearance of the deepest high amplitude reflectors on the vertical seismic data. The major results are given in Fig. 3. They include



Fig. 1 Location of the 'ECORS Bay of Biscay' profile and main structural features: 1, pre-Mesozoic; 2, oceanic crust; 3, bathymetry in metres; 4, limit of early Cretaceous in Parentis Basin; 5, Cap-Ferret (1) and Cap-Breton (2) depressions; 6, ECORS profile.

a simplified line drawing, a geological depth cross-section taking into account the ESP results, and gravimetric modelling. The features of the sedimentary cover had already been known for 20 yr from petroleum exploration (several tens of thousands of kilometres of conventional seismic profiles and 20 petroleum boreholes)⁶⁻⁹.

The Hercynian basement ranges in age between 400 and 300 Myr and includes older terrains. Our line runs over the southern segment of the Variscan fold belt. Numerous horizontal and north dipping reflections are visible between 9 to 18 km in the northern part of the line (Fig. 3b). These may be interpreted as Palaeozoic sediments (or meta sediments) beneath Variscan overthrusts or as slices of a previous lower crust which were stacked and overthrust to the south during the Variscan orogeny. We prefer, however, the second interpretation because of the similarity between these reflections and those observed commonly in the autochthonous lower crust. The Moho and the lower crust beneath this zone look as if they had remained undisturbed; it is likely that the deep roots of these thrusts must be found further north in a more internal zone of the fold belt. The scarce data gathered from oil wells on the supposed Palaeozoic foreland basin show low grade metamorphosed or non-metamorphic Palaeozoic sediments of unknown thickness in the Aquitaine region. Transparent on the seismic data, they have been included in the geological interpretation as part of the upper crust. Beneath the southern extremity of the Armorican Shelf and the Landes High, ESPs have depicted relatively low-velocity zones which can be clearly correlated with such series.

Continental extension characterized the Bay of Biscay margin during the Mesozoic¹⁰. An earlier extension which occurred during the Triassic and Lias broke up the Hercynian basement and initiated the intracontinental Aquitaine Basin. This was the result of the rifting event that preceded the opening of the central Atlantic¹¹. From the Kimmeridgian to the Aptian, rifting and subsidence of the northern continental margin of the Bay of Biscay is already known from previous work^{10,12,13}. Within the Parentis Basin, this profile shows that the rifting was accompanied by very extensive crustal thinning. The development of this half graben was particularly intense during this short time period with very rapid infilling by clastic sediments. The opening of the Bay of Biscay started in the late Aptian-earliest Albian times (~110 Myr) with the formation of an oceanic crust. Almost immediately after (early Albian), as shown by industrial seismic

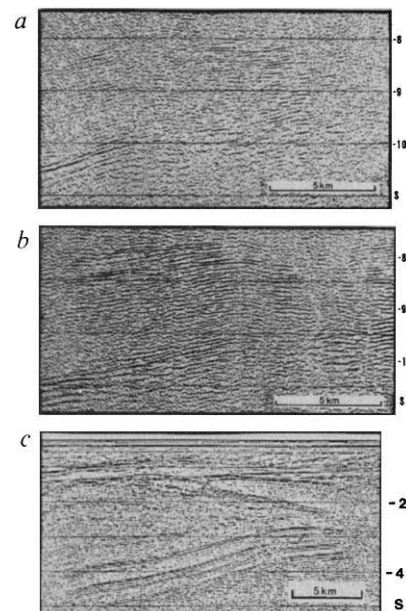


Fig. 2 Comparison of unmigrated near-offset (a) and large-offset data (b) showing the Moho and the layered lower crust; c, migrated near-offset data showing the rotated then inverted Mesozoic cover.

profiles, the predominantly east-west normal faults were rejuvenated as strike-slip faults⁹. This can be related to the east-west middle Cretaceous shortening event identified in western Europe^{14,15} and/or local compression along the plate boundary between Europe and Iberia. Subsidence was still active with important localized Albian depocentres. A second phase of shortening, but oriented broadly north-south, occurred during the late Cretaceous to Eocene in connection with the Pyrenean orogeny. The basin was then strongly inverted a second time with the development of large wavelength anticlinoria and, locally, reverse faults. The thick Triassic evaporites, already mobilized during the rifting phase, participated in the deformation of the series during the inversion.

The result of Pyrenean orogeny is especially clear in the last southern kilometres of the profile where a thin-skinned north-vergent tectonics corresponds to the Pyrenean deformation front. It mainly affected Eocene layers over the basement and its thin Mesozoic cover. Indeed, a few kilometres deeper inside the onshore Pyrenean range, the Palaeozoic and Mesozoic are also implicated in the thrusts. At this southern end of the profile, an important Moho deepening and thickening of the high velocity lower crust are observed. In this area, Hercynian thrust planes may have been reactivated during the Pyrenean orogeny. The Moho and the bottom of Mesozoic series have a conspicuous parallelism in the last third of the profile. This resemblance can be explained by a flexure of the crust because of the overloading of the nappes.

This profile emphasizes the very short-range variability of the deep crustal structure beneath different tectonic domains. The most spectacular feature is the very great crustal thinning beneath the rifted Parentis Basin. This crustal thinning is inferred from the reflection data with the abrupt disappearance of the layered lower crust on a 60-km width and with the almost total absence of the upper crust (Fig. 3a), and from the results of the ESP interpretation⁵. Although gravity data are scarce on the Aquitaine shelf, the gravity map¹⁶ shows a +40 mgal anomaly where our line crosses the east-west cylindrical Parentis Basin. The simple two-dimensional gravimetric modelling (Fig. 3c) that we performed to confirm the uprising of the Moho is consistent with the data observed, while the hypothesis of a continuous flat Moho would imply an important mismatch (at least 70 mgal assuming no drastic change in crustal densities).

The presence of a thin crust below the Parentis Basin must be related to the proximity of an oceanic domain because the rifted Parentis Basin is a failed arm of the oceanic Bay of Biscay (Fig. 1). Strike-slip movements and crustal shortening might

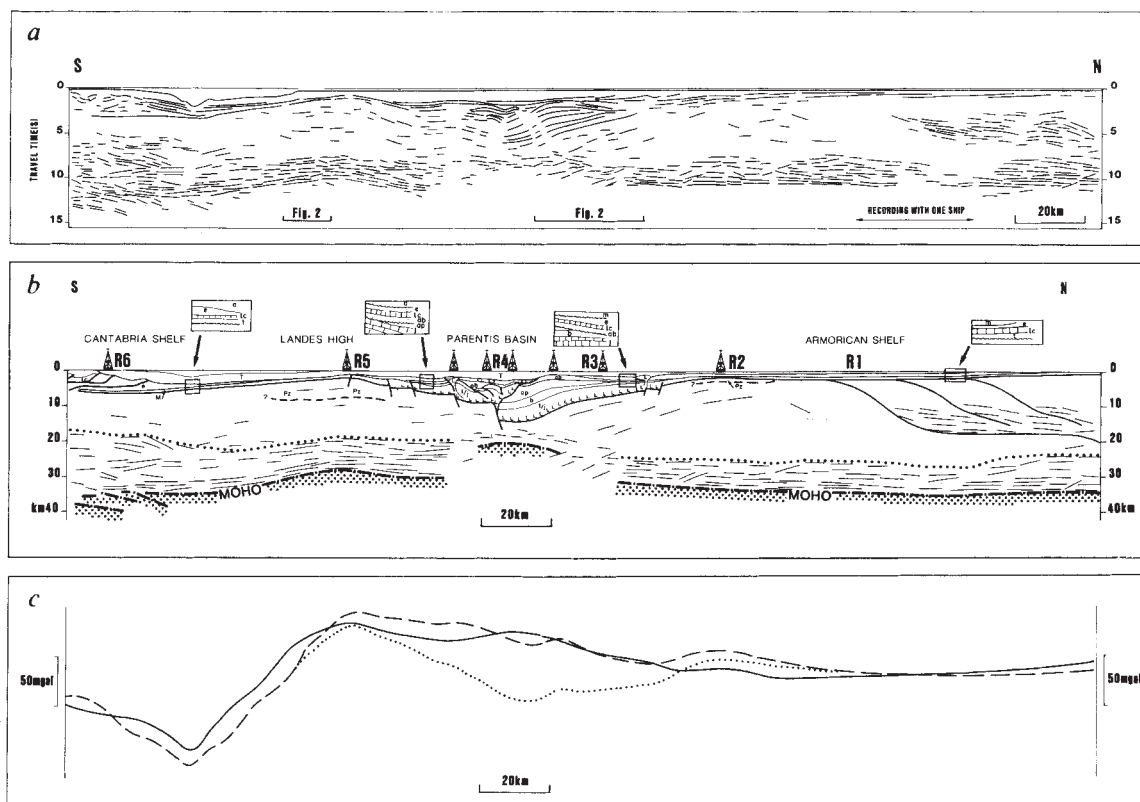


Fig. 3 Interpretation of the 'ECORS Bay of Biscay' profile: *a*, two-way time simplified line-drawing: the locations of selected sections shown in Fig. 2 are indicated at the bottom of the profile. *b*, Geological interpretation: the section is in km and is true-scale. The locations of ESPs (R1 to R6) and petroleum boreholes are indicated at the top. T: Tertiary, M: Mesozoic, Pz: Palaeozoic sedimentary series, O: Oligocene, e: Eocene, lc: Cretaceous, ab: Albian, ap: Aptian, b: Barremian, t/j: undifferentiated Triassic and Jurassic. *c*, Gravimetric modelling drawn from Fig. 3*b*. The specific densities used are: water: 1, Tertiary: 2.2, Mesozoic: 2.5, upper crust: 2.7, lower crust: 2.8, upper mantle: 3.3. Two models have been computed: the dashed line assumes mantle updoming below the Parentis Basin and the dotted line a flat Moho. The continuous line represents the data observed.

have partly contributed to the present image of the deep structure of the basin, but only three-dimensional mapping of the thinned crustal zone could document their interaction with the disappearance of the lower crust. However, it is clear that there are serious discrepancies between the amounts of horizontal extension by faulting and the total crustal thinning. Many studies made on such extensional basins have also shown that the superficial extension ratio measured cannot fully explain total crustal thinning below these basins or passive margins^{17,18}.

At present, three different conflicting hypotheses are offered to explain the formation of extensional sedimentary basins. First, the simple-shear model proposed by Wernicke is based on detachment faults which cut across the entire lithosphere^{19,20}. It takes into account frequent asymmetry of the rifted basins and implies mass conservation. Derived models have also been proposed suggesting that the crustal shear zone could run horizontally to the crust-mantle boundary or to the brittle-ductile transition²¹. Recent interpretation of deep seismic data around England have been proposed to support this model. On the Biscay profile (Fig. 4*a*) the lack of low-angle normal faults and, more severely, the location and geometry of the thinned zone make it difficult to apply the simple-shear model for the formation of the rifted Parentis Basin.

In the second hypothesis, thermo-mechanical models are based on mantle plume ascent initiated by a thermal disturbance^{22,23}. The resulting mantle displacement induces stretching at the base of the crust and causes ductile flow in the crust. If strongly temperature- and pressure-dependent rheologies are assumed for the upper and lower crusts²⁴, both crustal layers can be thinned by different amounts; their boundary corresponds to a certain physical state and not to a particular composition. Such models are derived from the stretching models proposed

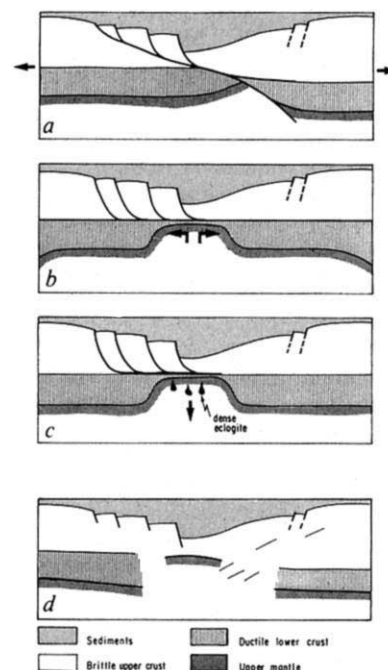


Fig. 4 Different models for the formation of the Parentis Basin with *a*, crustal detachment fault; *b*, ductile lower crust flow; *c*, non-conservation of the crustal mass. The data observed are shown in *d*, (see discussion in the text).

initially by McKenzie²⁵ (with uniform stretching) and Royden and Keen²⁶ (with non-uniform stretching). The brittle-ductile boundary may move upwards in the axis of the basin. This type of model can explain the discrepancy between the small superficial extension and the substantial thinning ratio (greater than 6) observed on our line (Fig. 4b).

Finally, in opposition to the previous models which all take into account crust mass conservation, non-conservation mass models are based on the gabbro-eclogite phase transformation. The density of the eclogitic facies is higher than the surrounding upper mantle. The crustal material which undergoes this phase change should sink in the mantle²⁷ (Fig. 4c). This transformation is commonly accepted in orogenic belts²⁸ but it is still a matter of discussion in extensional sedimentary basins because an eclogitic facies is expected to be stable only under high pressures²⁹. The southward dipping reflections visible on the northern edge of the basin (Fig. 4d) give support to the idea of crustal loss by sinking material in the upper mantle. On the other edge of the basin, the continuity between the top of the layered lower crust below the Landes High and the uprised Moho below the Parentis Basin could also suggest intrusion of mantle material into the lower crust and its gradual assimilation into the upper mantle³⁰ with deep crustal metamorphism. Such processes would cause an upward displacement between a pre-rift Moho and a new post-rift one.

Received 13 August; accepted 8 December 1986.

- McGeary, S. & Warner, M. R. *Nature* **317**, 795-797 (1985).
- BIRPS & ECORS *J. geol. Soc.* **143**, 45-52 (1986).
- Cazes, M. *et al. Nature* **323**, 144-147 (1986).
- DEKORP Research Group *J. Geophys.* **57**, 137-163 (1985).
- Marillier, F. *et al.* (in preparation).
- Cumelle, R., Dubois, P. & Seguin, J. C. *Phil. Trans. R. Soc. A* **305**, 63-84 (1982).
- Winnock, E. in *Histoire Structurale du Golfe de Gascogne*, paper IV.1 (Technip, Paris, 1971).
- Dardel, R. A. & Rosset, R. in *Histoire Structurale du Golfe de Gascogne*, paper IV.2 (Technip, Paris, 1971).
- Mathieu, C. *Bull. Cent. Rech. explor. Prod. Elf-Aquitaine* **10**, 33-47 (1986).
- Boillot, G. *et al.* in *Les Marges Continentales Actuelles et Fossiles Autour de la France* (Masson, Paris, 1984).
- Olivet, J. L. *et al.* in *Cinématique de l'Atlantique Nord et Centrale* (rapp. CNEXO N°54, 1984).
- Montadert, L. *et al.* in *The Geology of Continental Margins*, 323-342 (Springer, New York, 1974).
- de Charpal, O. *et al. Nature* **275**, 706-711 (1978).
- Benard, F. *et al. C. r. heb. Séanc. Acad. Sci., Paris*, **300**, 765-768 (1985).
- Tremolieres, P. *Rev. IFP*, **36**, 579-583 (1981).
- Lalaut, Ph., Sibuet, J. C. & Williams, C. *C. r. heb. Séanc. Acad. Sci., Paris* **292**, 597-600 (1981).
- Chenet, P. Y. *et al. AAPG-Memo*, **34**, 703-715 (1983).
- Le Douaran, S., Avedik, F. & Burrus, J. *Marine Geol.* **55**, 325-345 (1984).
- Wernicke, B. *Can. J. Earth Sci.* **22**, 108-125 (1985).
- Boillot, G. *et al. Bull. Cent. Rech. explor. Prod. Elf-Aquitaine* **10**, 95-104 (1986).
- Barbier, F., Duvergé, J. & Le Pichon, X. *Bull. Cent. Rech. explor. Prod. Elf-Aquitaine*, **10**, 105-121 (1986).
- Fleitout, L. & Yuen, D. *J. geophys. Res.* **89**, 9227-9244 (1984).
- Moretti, I. & Froidevaux, C. *Tectonics* **5**, 501-511 (1986).
- Moretti, I. *Tectonophysics* (in the press).
- McKenzie, D. P. *Earth planet. Sci. Lett.* **40**, 25-32 (1978).
- Royden, L. & Keen, C. E. *Earth planet. Sci. Lett.* **51**, 343-361 (1980).
- Artyuskov, E. & Baer, M. *Tectonophysics* **108**, 193-228 (1984).
- Richardson, S. & England, P. *Earth planet. Sci. Lett.* **42**, 183-190 (1979).
- Ringwood, A. in *Composition and Petrology of the Earth's Mantle* (McGraw-Hill, London, 1975).
- Ziegler, P. A. *Nature* **304**, 561 (1983).

Fate of superoxide in coastal sea water

Robert G. Petasne & Rod G. Zika

Rosenstiel School of Marine and Atmospheric Science, Division of Marine and Atmospheric Chemistry, University of Miami, 4600 Rickenbacker Causeway, Miami, Florida 33149, USA

The superoxide anion (O_2^-) is a key intermediate in oxygen redox chemistry, and may mediate many chemical transformations in the ocean. The photochemical formation of hydrogen peroxide in sea water has been postulated¹ to result from the disproportionation of superoxide, and here we report the results of a study which used the formation of H_2O_2 to characterize the dynamics of superoxide in coastal sea water. Midday rates of superoxide generation averaged $\sim 5 \times 10^{-7} \text{ mol l}^{-1} \text{ h}^{-1}$. In addition to rapid disproportionation to hydrogen peroxide, a substantial fraction of the superoxide flux was shunted off through unknown pathways; 24-41% of the superoxide flux did not lead directly to H_2O_2 formation. Our calculations predicted accelerated decomposition of superoxide in natural sea water relative to pure water; nevertheless, superoxide should be a relatively long-lived transient in sea water. We calculate the 50% decay time of a $10^{-8} \text{ mol l}^{-1}$ steady-state level to be 20 min.

The primary pathway for superoxide in aqueous solution is its rapid disproportionation to H_2O_2 and O_2 : $2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. Photochemically generated H_2O_2 is ubiquitous in the upper layers of the ocean^{2,3}. Superoxide, probably a common precursor to H_2O_2 for both thermal and photochemical source processes, could be an important chemical transient species in surface sea water. This reduced oxygen species has oxidizing, reducing and nucleophilic potential⁴⁻⁶. In air-saturated sea water, where most elements exist in their upper oxidation states, O_2^- provides an important and unique mechanism for reducing various chemical species. Its potential effect on organic substrates and transition metals may have biogeochemical significance in the marine environment.

Light-initiated electron-transfer processes involving O_2 , organic substrates, and transition metals have been postulated to generate O_2^- in sea water¹. Studies using the specific probe superoxide dismutase (SOD) have been used to test for the

presence of O_2^- in irradiated natural waters^{7,8} and aqueous solutions of environmentally relevant organic compounds⁹. However, these studies have not examined the fate of O_2^- and its significance in mediating chemical and biological processes in these natural environments. We have examined general reaction pathways and have made estimates of concentrations and lifetimes of O_2^- in sea water.

Previous estimates based on H_2O_2 measurements suggest O_2^- concentrations of $\sim 10^{-8} \text{ mol l}^{-1}$ in sea water¹⁰. This exceeds estimated intracellular levels by 3 or 4 orders of magnitude¹¹. To determine its lifetime in sea water, the disproportionation rate, as a first approximation, was assumed not to be affected by catalysts such as trace metals¹² due to their low activity in sea water. In addition, O_2^- should be considerably stabilized by the relatively high concentrations of the cations Ca^{2+} and Na^+ in sea water¹³. Recent extrapolations of these measurements to sea water media predict a three-fold decrease of the disproportionation rate¹⁴. A disproportionation rate constant (k_d) of $1.67 \times 10^4 \text{ mol}^{-1} \text{ l s}^{-1}$, adjusted for sea water pH = 8.0 and ionic interactions was used. Applying the above assumptions, lifetimes of O_2^- in sea water were calculated. The 50% decay of $10^{-8} \text{ mol l}^{-1}$ O_2^- was estimated to be ~ 100 min, in sea water assumed to be free from catalytic agents. These crude calculations suggest that a high steady-state level of O_2^- is possible in sea water, and provide an incentive for characterizing the transient.

The validity of these estimates was examined in the laboratory. The close coupling between O_2^- and H_2O_2 suggest that measurements of the relatively stable transient, H_2O_2 (60 h half-life in 0.2 μm filtered Biscayne Bay sea water), could be an excellent probe for O_2^- in sea water. H_2O_2 was measured¹⁵ during irradiation of coastal sea water samples collected from Biscayne Bay in Miami, Florida. Samples were irradiated with sunlight during midday in 300- cm^3 quartz round-bottom flasks, or with a 1,000-W Xe solar simulator in a 10-cm pathlength quartz cylindrical cell. In both experimental setups, H_2O_2 exhibited a linear increase relative to radiation flux (see Fig. 1), indicating that a steady-state concentration of O_2^- probably existed for all but the very early part of the experiment. To test the stability of H_2O_2 during irradiation its concentration was increased to $10^{-7} \text{ mol l}^{-1}$ before initiating the irradiation. A plot of H_2O_2 increase against radiation exposure for this solution was linear and had the same slope as a sample without the initial H_2O_2 addition. This indi-

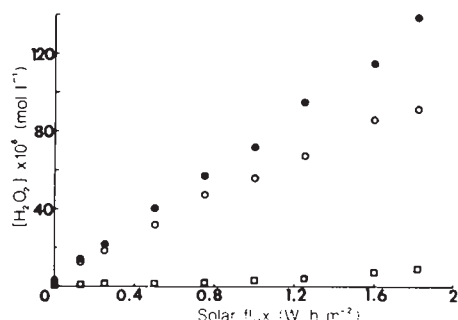


Fig. 1 H_2O_2 concentration as a function of solar flux. $\circ$, Coastal sea water sample filtered through a 0.2- μm polycarbonate membrane filter; $\bullet$, filtered coastal sea water in the presence of 2×10^{-7} M SOD; $\square$, aged ultraviolet-irradiated Gulf Stream water in the presence of 2×10^{-7} M SOD.

cates that no significant first or higher order H_2O_2 decomposition reactions are active during the irradiation period. This apparent photochemical and thermal stability of H_2O_2 during these short duration experiments reinforced its value as a probe for O_2^- .

Low steady-state levels of O_2^- were expected to persist after removal from light. The precision of our analytical method (2%) would not allow us to detect small increases in H_2O_2 concentration due to the slow disproportionation of these relatively lower levels of O_2^- . Because H_2O_2 concentrations reached 10^{-6} mol l^{-1} during irradiation, a subsequent increase of 10^{-8} mol l^{-1} H_2O_2 in the dark would be undetectable using our analytical method. This observation puts an upper limit of $\sim 1\text{--}2 \times 10^{-8}$ mol l^{-1} on the O_2^- steady-state concentration in irradiated coastal sea water, consistent with previous estimates¹⁰. On the other hand, the possibility that higher concentrations of O_2^- exist and are rapidly consumed just before the H_2O_2 analysis is unlikely. Unless there are efficient non- H_2O_2 producing O_2^- destruction pathways operating in the irradiated sample, these high levels of O_2^- would result in much higher steady-state concentrations of H_2O_2 , contrary to extensive field measurements^{2,3}.

In addition to H_2O_2 , the enzyme SOD is a valuable tool in characterizing O_2^- in sea water. SOD dramatically increases the disproportionation rate of O_2^- ($k_{\text{SOD}+\text{O}_2^-} = 2 \times 10^9$ mol l^{-1} s $^{-1}$)¹⁶, resulting in enhanced H_2O_2 production if there are other non-disproportionation pathways¹⁷. Although the catalytic rate constant may be reduced by an order of magnitude due to ionic strength effects^{18,19} (ionic strength = 0.7 mol l^{-1} , $k_{\text{SOD}+\text{O}_2^-} = 10^8$ mol l^{-1} s $^{-1}$), SOD should still be a useful scavenger of O_2^- in competition studies in sea water. Figure 1 shows enhanced photochemical production of H_2O_2 in coastal sea water in the presence of 2×10^{-7} mol l^{-1} SOD (SOD 3,000 units mg^{-1} , MW 32,600). The reported inactivation of SOD by H_2O_2 (ref. 20) did not apply at the relatively low levels of H_2O_2 accumulated

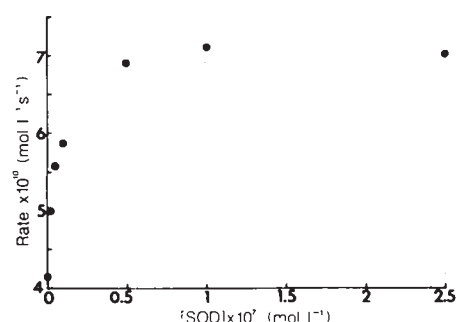
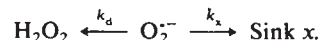


Fig. 2 H_2O_2 production rate as a function of SOD concentration in filtered coastal sea water exposed to a 1,000 W Xe solar simulator. The production rate was determined as the rate of increase of H_2O_2 concentration as a function of irradiation time.

in our experiments. In addition, experiments in which H_2O_2 was added to SOD-containing samples showed that catalase or peroxidase activity of the SOD during the experiment was negligible. Further tests of SOD in organic-free sea water showed that its activity was not lost and that it did not contribute significantly to H_2O_2 production during sample irradiation (see Fig. 1).

The data in Fig. 1 lead to another important conclusion, that a significant fraction of O_2^- does not lead to H_2O_2 . This conclusion is based on the fact that SOD increases the disproportionation rate in sea water by four orders of magnitude, thus rerouting any O_2^- which might have been consumed through other pathways. In the absence of SOD, a significant fraction of O_2^- in coastal seawater does not lead directly to the H_2O_2 pool:



This substantiates superoxide's dual role in coastal sea water as a precursor to H_2O_2 and as a chemical initiator of other reactions.

Quantitative evaluation of this branching is valuable in assessing the relative importance of distinct reaction pathways of O_2^- in sea water. Although SOD scavenges O_2^- rapidly, species exist in coastal sea water which outcompete the SOD for O_2^- . It is possible to estimate the upper limit of production rates by measuring H_2O_2 in the presence of successive increments of SOD until the rates level off (see Fig. 2). The plateau in Fig. 2 indicates that SOD has outcompeted all other scavengers in coastal sea water for O_2^- . At the maximum rate obtained, the entire O_2^- flux should have been converted to H_2O_2 .

Thus, the O_2^- production rate ($P_{\text{O}_2^-}$) was calculated from this maximum H_2O_2 production rate. H_2O_2 production rates ($P_{\text{H}_2\text{O}_2}$) in the absence of SOD and in the presence of maximum SOD concentrations were used to calculate O_2^- branching ratios (Q).

Table 1 H_2O_2 production rates, O_2^- production rates, O_2^- steady-state concentrations, pseudo first-order rate constants for the unknown O_2^- sink, and branching ratios for several coastal sea water samples exposed to midday sunlight or a 1,000 W Xe solar simulator

Date	Sample Source	Absorbance	$P_{\text{H}_2\text{O}_2}$ (10^{11} mol l^{-1} s $^{-1}$)	$P_{\text{O}_2^-}$ (10^{10} mol l^{-1} s $^{-1}$)	$[\text{O}_2^-]$ (10^8 mol l^{-1})	k_x^* (10^4 s $^{-1}$)	Q
27 May 1985	22N15 Sun	0.1342	6.2	1.7	8.6	5.4	0.28
25 October 1985	22N16 Sun	0.1540	6.2	1.6	8.6	4.6	0.24
30 December 1985	22N18 Sun	0.0952	2.5	0.78	5.5	5.0	0.35
30 December 1985	22N18 Lamp	0.0952	41.0	14.0	22.0	26.0	0.41

Sunlight-induced rates were converted to time dependent rates by assuming a typical midday summer value of 0.45 W-h m^{-2} per h of sunlight exposure. Sample collection date, optical absorbance at 300 nm in a 10-cm cell, and light source are indicated. $P_{\text{O}_2^-} = 2P_{\text{H}_2\text{O}_2}$ at maximum [SOD]; $[\text{O}_2^-] = (2P_{\text{H}_2\text{O}_2}/k_d)^{0.5}$ without SOD; $k_x^* = (P_{\text{O}_2^-} - P_{\text{H}_2\text{O}_2})/[\text{O}_2^-]$ without SOD; $Q = (P_{\text{O}_2^-} - 2P_{\text{H}_2\text{O}_2})/P_{\text{O}_2^-}$ without SOD.

Steady-state levels of O_2^- and composite pseudo first-order rate constants for the unknown reactions ($k_x^* = k_x[X]$) were calculated assuming a k_d of $1.67 \times 10^4 \text{ mol l}^{-1} \text{ s}^{-1}$ (ref. 14). Table 1 shows these results for several coastal sea water samples in different experimental conditions. Although absolute values of rates and steady-state concentrations may not reflect the real situation due to the artificial experimental setups and assumptions of k_d , the data have important implications.

Based on H_2O_2 production a considerable fraction of O_2^- was being consumed in other undefined processes. Superoxide in aqueous solution reacts with a variety of substrates with rate constants $\sim 10^8\text{--}10^9 \text{ mol l}^{-1} \text{ s}^{-1}$ (ref. 6). Therefore, values of k_x^* imply that numerous trace species present at environmental concentrations ($10^{-10}\text{--}10^{-13} \text{ mol l}^{-1}$) are potential scavengers of O_2^- in sea water. The generally oxidized state of sea water constituents suggests that O_2^- functions predominantly as a reducing agent in surface sea water.

To estimate the lifetime, the O_2^- decay was described by a two-term rate equation including k_d and k_x^* ($d[O_2^-]/dt = k_d[O_2^-] + k_x^*[O_2^-]$). The assumed k_d and the calculated k_x^* were combined into an integrated rate equation to yield an estimate of O_2^- lifetime in sea water. A 50% loss of $10^{-8} \text{ mol l}^{-1} O_2^-$ occurred after 20 min in sea water compared with the previous estimate of 100 min in distilled water, allowing for ionic interactions. Although accelerated decomposition of O_2^- is expected

in sea water, it still seems to be a relatively long-lived transient.

This research was supported by the Office of Naval Research grant N00014-80-C-0042. We thank Drs E. S. Saltzman, J. Plane, K. Mopper and A. Fischer for useful comments.

Received 30 June; accepted 27 October 1986.

1. Zika, R. G. thesis, Dalhousie Univ. (1978).
2. Zika, R. G., Moffett, J. W., Petasne, R. G., Cooper, W. J. & Saltzman, E. S. *Geochim. cosmochim. Acta* **49**, 1173-1184 (1985).
3. Zika, R. G., Saltzman, E. S. & Cooper, W. J. *Mar. Chem.* **17**, 265-275 (1985).
4. Sawyer, D. T. & Valentine J. S. *Acet. chem. Res.* **14**, 393-400 (1981).
5. Bielski, B. H. J. in *Gas-Liquid Chemistry of Natural Waters* Vol. 1 (ed. Newman, L.) (Brookhaven Nat. Lab., New York, 1984).
6. Bielski, B. H. J., Cabelli, D. E., Arudi, R. L. & Ross, A. B. *J. phys. chem. ref. Data* **14**, 1041-1100 (1985).
7. Cooper, W. J. & Zika, R. G. *Science* **220**, 711-712 (1983).
8. Baxter, R. M. & Carey, J. H. *Nature* **306**, 575-576 (1983).
9. Draper, W. M. & Crosby, D. G. *J. agric. Fd Chem.* **31**, 734-737 (1983).
10. Zafiriou, O. C., Jousot-Dubien, J., Zepp, R. G. & Zika, R. G. *Envir. Sci. Technol.* **18**, 358A-371A (1984).
11. Chance, B., Sies, H. & Boveris, A. *Physiol. Rev.* **59**, 527-605 (1979).
12. Bielski, B. H. J. & Allen, A. O. *J. phys. Chem.* **81**, 1048-1050 (1977).
13. Bray, R. C., Mautner, G. N., Fielden, E. M. & Carle, C. I. in *Superoxide and Superoxide Dismutases* (eds Michelson, A. M., McCord, J. M. & Fridovich, I.) 61-75 (Academic, New York, 1977).
14. Millero, F. J. *Eos* **66**, 1257 (1986).
15. Zika, R. G. & Saltzman, E. S. *Geophys. Res. Lett.* **9**, 231-234 (1982).
16. Klug, D., Rabani, J. & Fridovich, I. *J. biol. Chem.* **247**, 4839-4842 (1972).
17. Weser, U. & Voelcker, G. *FEBS Lett.* **22**, 15-18 (1972).
18. McAdam, M. E. *Biochem. J.* **161**, 697-699 (1977).
19. Cudd, A. & Fridovich, I. *J. biol. Chem.* **257**, 11443-11447 (1982).
20. Bray, R. C. *et al. Biochem. J.* **139**, 43-61 (1974).

Annual variation of heat transport in the Pacific and Indian Oceans

Jane Hsiung, Reginald E. Newell & Thomas Houghtby

Room 54-1517, Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

An important element in the maintenance of the Earth's climate is the redistribution of heat from surplus to deficit regions. Previous estimates¹⁻⁵ have shown that the oceanic heat transport is comparable to that of the atmosphere especially in the tropics and subtropics and that the ocean shows a similar seasonal transport carrying energy into the winter hemisphere. The annual mean meridional heat transport in individual oceans has also been estimated⁶⁻⁸. Heat storage can be computed from subsurface data to provide a picture of the annual cycle of the heat transport for each ocean separately. Lamb^{9,10} presented such estimates for the Atlantic Ocean and found a southward heat transport in the November-December season with a northward heat transport in both hemispheres at other seasons. A similar method is used here to compute the seasonal variation of the heat transport north of 20°S for both the Pacific and the Indian oceans. The Pacific Ocean exhibits two regimes. North of 20°N transport is mostly northward with a maximum in September. Transport between 20°N and 20°S is evenly divided between northward (with a March maximum at 10°N) and southward (with an August maximum at 10°S). In the Indian Ocean, the transport is mostly southward except during January-February when a small northward transport appears north of 5°S .

The computation of the seasonal meridional heat transport is based on the theory that in the long-term mean, the oceanic surface-energy balance of a column is equal to the sum of the rate of heat-content change and the divergence of oceanic heat transport. Surface-energy balance and the rate of heat-content change can both be computed from marine observations. The divergence of oceanic heat transport can then be inferred as the difference between these two quantities. With an appropriate boundary condition, heat-transport values for each latitude can be computed by successive integration of the zonal average of the divergence of heat transport.

The surface-energy balance is calculated using the so-called bulk formulations with ship data from the Consolidated Data Set. Heat-content data are calculated from temperature profiles from 0 to 300 m using observations from the Master Oceanographic Observations Data Set. Both data sets are from the US Navy's Fleet Numerical Oceanographic Center. Monthly averages for a 31-yr data period, 1949-79, were performed on a spatial resolution of $5^\circ \times 5^\circ$. Details of the surface-energy calculation are presented elsewhere¹¹; results have been used to estimate annual meridional heat transport in both oceans⁷. Monthly heat contents for the Pacific and Indian oceans were calculated for layers 0-50 m, 50-100 m, 100-150 m, 150-200 m and 200-300 m if a minimum of three observations existed for that layer within the $5^\circ \times 5^\circ$ grid. A climatology of the heat content for the 0-300 m layer was then made from the individual layers. Several iterations were made to filter out observations >4 standard deviations away from the mean. Monthly (calendar) heat-content changes were computed using a centred-difference scheme (January heat-storage change were computed from the difference between February and December mean heat storages). Details of the heat-storage computation will be presented elsewhere. The latitudinal distribution of the number of soundings used for heat-storage calculation is presented in Table 1.

Previous estimates of Pacific Ocean annual heat transport have shown that the direction of transport is largely poleward^{6,7}, however, due to the uncertainties involved in surface-energy flux estimates, there are some doubts as to the direction of the transport in the North Pacific¹². Recent estimates made from direct measurement of the ocean's velocity and temperature at 32°N during April-June have shown a northward transport¹³. Bryan¹⁴ found a southward transport of the magnitude $1.1 \times 10^3 \text{ TW}$ at 32°N . He claims that mesoscale eddies along the Kuroshio may be important in compensating the large-scale heat transport. Figure 1 shows the annual variation of the heat transport in the Pacific Ocean obtained from this study. The boundary condition assumed here is that transport is zero at 60°N . Values are shown only for north of 20°S because data south of 20°S are extremely sparse both in the heat-content calculation and in the surface-energy flux calculation. One feature worth noting in Fig. 1 is that the annual cycles are quite different north and south of 20°N . North of 20°N , the transport is mostly northward with a broad maximum centred around

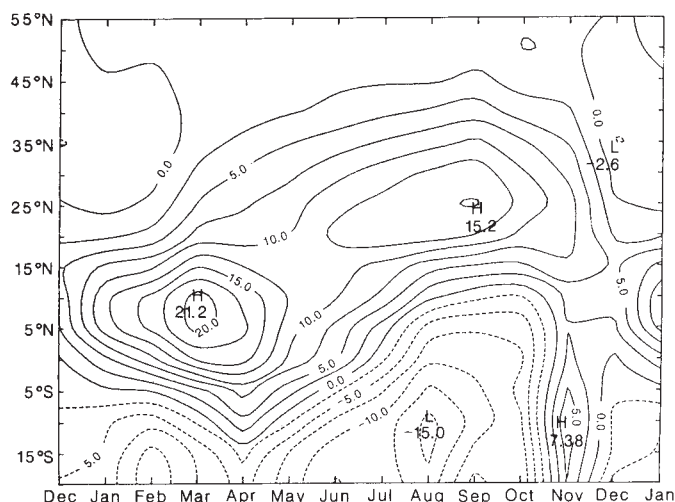


Fig. 1 Meridional heat transport for the Pacific Ocean in units of 10^2 TW. Positive value denotes northward transport. The non-continental western boundary for the Pacific Ocean extends from Darwin along the Indonesian chain into the Malaysian Peninsula and southwards from Tasmania along 150° E.

September. South of 20° N, there is a maximum in northward transport in March at 10° N and a maximum in southward transport in August at 10° S. The amplitude of the annual cycle is quite large, ranging from 2.6×10^3 TW at 5° N to $\sim 5 \times 10^2$ TW at 40° N. The annual amplitude in the subtropical regions is similar in both the Atlantic¹⁰ and the Pacific oceans, but the Atlantic has a much larger annual cycle in the high northern latitudes and in the Southern Hemisphere. At 25° N, the annual cycle is 1.7×10^3 TW in the Atlantic and 1.5×10^3 TW in the Pacific. At 50° N the annual cycle is 1.4×10^3 TW in the Atlantic Ocean and 2×10^2 TW in the Pacific. At 15° S, it is 3.6×10^3 TW in the Atlantic Ocean and 2.2×10^3 TW in the Pacific. The general feature of the heat transport in the Pacific Ocean is similar to the global oceanic heat transport given by Oort and Vonder Haar³, with negligible transport north of 45° N and a maximum northward transport at 10° N during March–April. However, the magnitude of their global transport is much greater than ours for the Pacific Ocean alone as would be expected.

Unlike the Atlantic Ocean, there are few direct estimates of heat transport in the Pacific Ocean north of 20° S with which our results may be compared except for those mentioned before. However, we can compare our observations with some modelling results. They have shown rather large seasonal changes in poleward heat transport in the Pacific Ocean^{15,16}. One model¹⁵ yields a maximum northwards flux of 1.2×10^3 TW at about 10° N in January with a maximum southwards flux of 1.3×10^3 TW at $\sim 5^\circ$ S in July, both comparable to the values of Fig. 1. This seasonal variation is thought mainly to be due to the Ekman transport term¹⁷ which varies with the zonal wind stress, although when the winds are weak in the summer it is thought that the equatorial geostrophic transport dominates¹⁶. Another model¹⁶ based only on wind-stress forcing gives the same general magnitude of the equatorial Pacific fluxes but shows maximum fluxes in February and September in the Northern Hemisphere. Note that the month-to-month variation of oceanic heat transport for the Pacific was first estimated from the models and has been confirmed from the data reported here.

The seasonal variation of heat transport in the Indian Ocean is shown in Fig. 2. The transport across 25° N is assumed to be zero as a boundary condition. With the exception of small northward transport between November and March, north of 5° S, the direction of the transport is southward. This agrees with results on the annual heat transport^{6,7} which indicated southward transport in all latitudes in the Indian Ocean. The northward heat transport in the winter months at higher latitudes is

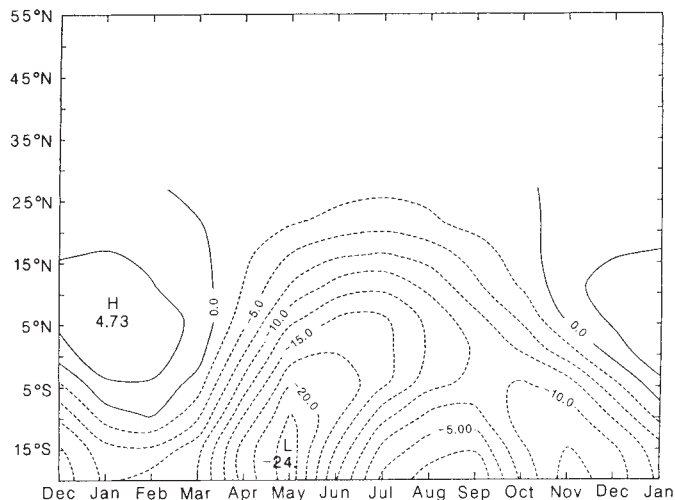


Fig. 2 Meridional heat transport for the Indian Ocean in units of 10^2 TW. Positive value denotes northward transport. The non-continental eastern boundary for the Indian Ocean is the same as the western boundary of the Pacific Ocean described in Fig. 1.

not surprising considering the large heat loss at 20 – 30° N in winter. Another interesting feature is the propagation of maximum southward heat transport from 20° S to 10° N during May–July. This could be associated with the onset of the Indian monsoon season. Comparing our results with those obtained by Hastenrath and Lamb¹⁸, our southward transport values are similar in the northern summer (1.0 compared with 0.8×10^3 TW). In the northern winter, they obtained a range of 1×10^2 TW northward to 1×10^2 TW southward transport, while we have a northward transport of 4×10^2 TW. Again, there are no direct estimates of seasonal transport north of 20° S in the Indian Ocean that could validate our findings.

Considerable uncertainties exist in the estimated meridional heat transport. Surface-energy flux estimates suffer not only from data inaccuracies but also from systematic errors from empirical formulae. Typical error estimates¹¹ given to this quantity are ~ 5 – 20 W m^{-2} . Errors associated with heat-content calculations stem mostly from data observation errors and biases. Assuming a 15 W m^{-2} error in both surface-heat flux and heat-storage change^{3,9–11} and that these errors are additive, the uncertainties of the transport at each latitude are calculated and presented in Table 1. The uncertainties get progressively larger

Table 1 Latitudinal distribution of soundings used for heat-storage calculations

Latitude	Indian Ocean		Pacific Ocean	
	No. of soundings	Uncertainties (10^{14} W)	No. of soundings	Uncertainties (10^{14} W)
55–60° N			1,010	0.67
50–55° N			1,279	1.6
45–50° N			2,189	2.8
40–45° N			3,746	4.2
35–40° N			7,952	5.8
30–35° N			18,672	7.7
25–30° N	153	0.07	11,167	8.8
20–25° N	1,516	0.3	15,696	11.1
15–20° N	1,006	0.8	8,667	13.8
10–15° N	1,934	1.7	8,781	16.7
5–10° N	2,065	2.6	2,533	19.9
0–5° N	1,569	3.6	1,802	23.1
0–5° S	1,397	4.7	1,053	26.0
5–10° S	723	5.9	787	28.9
10–15° S	677	7.5	844	31.6
15–20° S	357	8.9	611	34.1

southward as the errors propagate with a north-to-south integration. It is assumed the errors at each latitude belt had the same sign whereas in reality there may be some cancellations. Therefore, the uncertainties should be treated as a maximum estimate. The question of whether the heat-content integration should be carried to a depth >300 m was considered. However, we decided that this would probably introduce more errors rather than reducing the uncertainties in the total heat content. A previous study¹⁹ showed that there is a good agreement between the 0–225 m layer and the 0–550 m layers in calculating oceanic heat-content changes. The zero-flux boundary conditions assumed at the northern boundary of the oceans are fairly good assumptions. In the Pacific Ocean, previous studies^{20,21} indicated a northward transport of 2 TW through the Bering Strait and it is unlikely that the transport across 60° N is much larger. In the Indian Ocean, the existence of land at 30° N makes our boundary condition a fairly safe one. Experiments were carried out using other estimates of surface-energy flux to see if the resulting transports differ significantly from the results obtained here. We used flux estimates from Clark²² in the North Pacific and from Hastenrath and Lamb²³ in the Indian Ocean. In the North Pacific Ocean, the magnitude of the transport is much greater with Clark's flux data. The maximum transport north of 20° N is 2.8×10^3 TW in July (instead of 1.5×10^3 TW in September). The direction of the transport remains the same. In the Indian Ocean, results show only slight changes in the transport results—the main features of the transport are the same. Future work on direct estimates of the heat transport in these two oceans may shed some light on our results presented here. Meanwhile, comparison of the models with these observational results may produce a better understanding of the important physics.

We thank Oliver J. Newell for computing the heat content from $>3 \times 10^6$ bathythermograph soundings, the NSF, Climate Dynamics Program (grant ATM-8517107) and the Department of Energy (contract DE-ACO2-76EV12195) for support. Cathy Smith made a summary of the heat content data with support from the MIT Undergraduate Research Opportunities Program (UROP). T.H. received partial support from UROP and an award from the MIT Sea Grant Program. We thank Roger Bauer of Compass Systems, Inc.

Received 15 June; accepted 7 November 1986.

1. Newell, R. E., Kidson, J. W., Vincent, D. G. & Boer, G. J. *The General Circulation of the Tropical Atmosphere and Interactions with Extratropical Latitudes*, Vol. 2 (MIT, Cambridge, 1974).
2. Vonder Haar, T. H. & Oort, A. H. *J. phys. Oceanogr.* **3**, 169–172 (1973).
3. Oort, A. H. & Vonder Haar, T. H. *J. phys. Oceanogr.* **6**, 781–800 (1976).
4. Newell, R. E. & Chiu, L. S. in *Climatic Variations and Variability: Facts and Theories* (ed. Berger, A.) 21–61 (Reidel, Dordrecht, 1981).
5. Carissimo, B. C., Oort, A. H. & Vonder Haar, T. H. *J. phys. Oceanogr.* **15**, 82–91 (1985).
6. Hastenrath, S. J. *J. phys. Oceanogr.* **12**, 922–927 (1982).
7. Hsiung, J. J. *J. phys. Oceanogr.* **15**, 1405–1413 (1985).
8. Hastenrath, S. J. *J. phys. Oceanogr.* **10**, 159–170 (1980).
9. Lamb, P. J. *Nature* **290**, 766–768 (1981).
10. Lamb, P. J. & Bunker, A. F. *J. phys. Oceanogr.* **12**, 1388–1410 (1982).
11. Hsiung, J. J. *geophys. Res.* **91**, 10585–10606 (1986).
12. Talley, L. D. *J. phys. Oceanogr.* **14**, 231–241 (1984).
13. Roemmich, D. in *Proc. of IAMAP/IAPSO Joint Assembly*, Honolulu (1985).
14. Bryan, K. J. *geophys. Res.* **67**, 3403–3414 (1962).
15. Bryan, K. & Lewis, L. J. *J. geophys. Res.* **81**, 2503–2517 (1979).
16. Pares-Sierra, A. F., Inoue, M. & O'Brien, J. J. *J. geophys. Res.* **90**, 3293–3303 (1985).
17. Bryan, K. J. *mar. Res.* **40**, 39–53 (1982).
18. Hastenrath, S. J. & Lamb, P. J. *J. phys. Oceanogr.* **10**, 694–708 (1980).
19. Levitus, S. J. *J. phys. Oceanogr.* **14**, 727–746 (1984).
20. Aagaard, K. & Greisman, K. J. *geophys. Res.* **80**, 3821–3827 (1975).
21. Hughes, F. W., Coachman, L. K. & Aagaard, K. *Oceanography of the Bering Sea*, 59–98 (Institute of Marine Science, University of Alaska, 1972).
22. Clark, N. E. thesis, Massachusetts Institute of Technology, (1967).
23. Hastenrath, S. J. & Lamb, P. J. *Climate Atlas of the Indian Ocean, Part II: The Oceanic Heat Budget* (The University of Wisconsin Press, Madison, 1978).

Marsupial from the earliest Late Cretaceous of Western US

Richard L. Cifelli* & Jeffrey G. Eaton†

* Department of Zoology and Stovall Museum, University of Oklahoma, Norman OK 73019, USA

† Museum and Department of Geological Sciences, Campus Box 315, University of Colorado, Boulder, Colorado 80309, USA

The divergence and earliest radiations of the two great groups of living mammals, marsupials and placentals, is presumed to have occurred sometime early in the Cretaceous¹. Knowledge of forms from this time period is therefore essential to understanding the evolutionary and biogeographic history of mammals. Marsupials and placentals are comparatively well known in the latest Cretaceous (Campanian, Maastrichtian), where they are represented in a relatively well documented succession of faunas in North America and (for placentals only) Asia^{2,3}. Before that time, various archaic presumed relatives⁴ are known from scattered North American, European and Asian localities of early Cretaceous age⁵. Recent discoveries in Asia^{6,7} seem to support an earlier suggestion¹ that placentals were already well established on that continent by the middle of the Cretaceous. Because of a persisting gap in the New World fossil record before the first appearance of unequivocal marsupials in the early Campanian⁸, the early history of marsupials has remained obscure. We describe here the oldest known marsupial mammal, from the earliest Late Cretaceous (Cenomanian) of western North America and discuss its implications for the origin and biogeography of marsupials.

Order Marsupialia Illiger, 1811
?Family Stagodontidae Marsh, 1889
Pariadens new genus

Type and only species: *Pariadens kirklandi*, new

Diagnosis: Similar to Stagodontidae in having lower molar para-

conid as large or larger than metaconid, but lacking the extreme enlargement of the paraconid (and its isolation by development of a carnassial notch) in known forms (*Didelphodon*, *Eodelphis*). The trigonid is less compressed than in known stagodontids; the trigonid cusps are relatively robust and, excepting *Glasbius* and *Roberthoffstetteria*, lower relative to the talonid than in other Cretaceous marsupials. The entoconid is the largest cusp of the talonid and is anteroposteriorly elongated, forming a salient lingual entocristid as in some pedomiids. The cristid obliqua originates behind the protoconid on M_2 and is moved more medial on M_{3-4} , unlike either Didelphidae or Stagodontidae. The molars increase in length and breadth posteriorly as in stagodontids, but unlike known pedomiids or didelphids.

Pariadens kirklandi, new species

Etymology: The generic name refers to the provenance of the holotype, which was found by the bank of the Paria River; the species is named for James Kirkland, who found the locality. **Type and only specimen:** UCM (University of Colorado Museum) 54155, part of left mandibular ramus preserving the last three molars, presumably M_{2-4} .

Locality and horizon: UCM Loc. 85358 (specific locality data on file at UCM), southwestern Utah; middle of the Dakota Formation, middle Cenomanian^{9,10}.

The teeth are complete, although postmortem shear has displaced the lingual side of each talonid superiorly; the first tooth of the series was most affected by this distortion, the last tooth the least. The molars increase in length and width from M_2 to M_4 (Table 1). An anterocingulid is moderately developed anterolaterally on each tooth, and the wear of this structure suggests that it served a shearing function. Unlike therians of metatherian–eutherian grade and most Cretaceous Eutheria, the talonids are as wide or wider than the trigonids and the trigonid to talonid height differential is moderate. The trigonid is open lingually with low and robust cusps; carnassial notches in the paracristid and protocristid have been obliterated by wear and, at most, were only weakly developed. The paraconid is more robust, with a broader base, than the metaconid on all three

Table 1 Dental measurements (mm) of *Pariadens kirklandi*

	Length	Trigonid width	Talonid width*
M ₂	2.92	1.63	1.56
M ₃	3.19	1.78	1.82
M ₄	3.82	1.87	1.73

* Measurements as defined by Lillegraven¹.

teeth; on M₂₋₃ paraconid is higher than metaconid and on M₄ they are subequal in height. The talonid is dominated by the entoconid, which is elongate and tall, especially on M₄, even allowing for the distortion noted above. The entoconid forms a sharp, elevated lingual wall. The hypoconulid, where visible (M₂, M₄), is small, lingually placed, and merged or "twinned" with the entoconid. A salient crest, obscure on M₂₋₃ because of tight appression of the tooth series, descends anterolabially from the hypoconulid to the base of the hypoconid. The cristid obliqua joins the trigonid behind the protoconid on M₂ and is moved lingually, joining the trigonid at a point inferior to the lowest point in the protocristid, on M₃₋₄.

The earliest unequivocal marsupials previously described were recovered from the early Campanian upper Milk River Formation of Alberta, Canada⁸. This fauna, the only known fauna of 'Aquilan' age¹¹ and the oldest well known Late Cretaceous mammal fauna in the world^{3,12}, includes seven marsupial species referable to three families, indicating that significant morphologic and taxonomic diversification among marsupials already had occurred⁸.

The affinity of *Holoclemensia texana*, from the late Early Cretaceous (Albian) Paluxy Formation of Texas, referred by its describer¹³ to the Marsupialia, is enigmatic. The species is currently excluded from the Marsupialia by most workers^{5,14-17} (but see Fox¹⁸), who have variously considered it either as a metatherian-eutherian grade mammal of uncertain affinities, too primitive to be ordinally placed, or as a representative of a divergent, early group of tribosphenic Theria. *Pariadens* is much larger than any known Trinity therian, and is not only fully tribosphenic but has a broad talonid basin.

The distinctiveness of the major groups of living therian mammals, Marsupialia and Eutheria, is readily apparent in comparisons of soft structures and particularly in reproductive processes^{1,19}. Distinction of primitive members of these groups from each other and from archaic tribosphenic therians^{20,21}, especially with the fragmentary materials generally available, is considerably less certain. Nonetheless, study of Late Cretaceous North American Theria has allowed establishment of dental criteria characteristic of, at least, Campanian and later forms^{17,22}. On this basis, and with reference to mammals of tribosphenic grade^{14,16} for outgroup comparison and establishment morphocline polarity, presumed synapomorphic features of marsupial lower molars include: 1, hypocunilid and entoconid closely approximated, 'twinned', and well separated from hypoconid; 2, paraconid in a lingual position, nearly in line with entoconid and metaconid; and 3, metaconid usually distinctly lower than protoconid and higher than or approximating paraconid in height^{17,22}. In addition, among Cretaceous forms, marsupials generally differ from placentals in having a reduced height differential between trigonid and talonid; a broadened talonid, subequal to or greater than the trigonid in width, particularly maintained on more posterior molars; and a postcingulid developed on posterobuccal angle of the tooth, from hypoconulid to base of hypoconid. With the exception of (3), this suite of characters is found in *Pariadens kirklandi*, which is accordingly referred to the Marsupialia.

More precise affinities of *Pariadens* within the Marsupialia are problematic. Known North American Cretaceous marsupials are conventionally placed in three families, Pedomyidae,

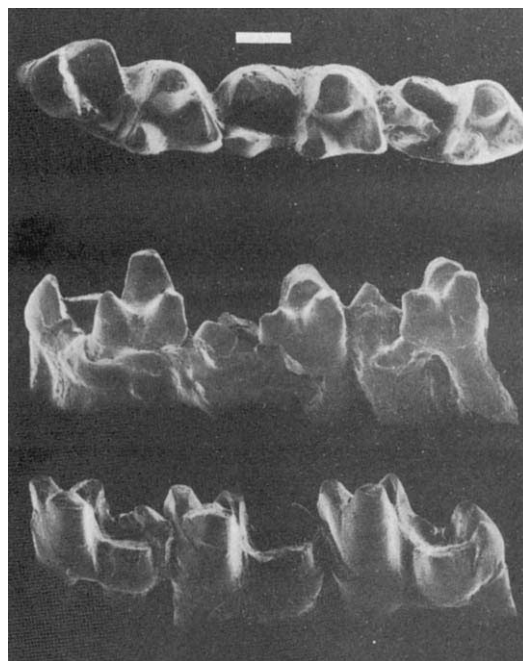


Fig. 1 M₂₋₄ of *Pariadens kirklandi* (UCM 54155) in occlusal (top), oblique lingual (middle), and oblique labial (bottom) views (the containing jaw fragment has been eliminated from the photographs). Scale bar, 1 mm.

Stagodontidae, and Didelphidae²³ (but see Marshall²⁴). The Didelphidae, the only family known to have survived into the Tertiary, is united largely on the basis of shared primitiveness; each of the other families is characterized by specialized morphology^{23,24}. *Pariadens* bears special resemblance to Stagodontidae in its large size, in the posteriorly increasing size of its molar series, and in the fact that the lower molar paraconid equals or exceeds the metaconid in size. We tentatively refer the genus to the Stagodontidae on this basis. *Pariadens* lacks, however, some characteristic features seen in lower molars of the latest Cretaceous genera *Didelphodon* and *Eodelphis*^{23,25}, including a labial attachment of cristid obliqua to trigonid on M₃₋₄, an enlarged anterocingulid, and compressed trigonid with well developed paraconid/paracristid carnassial notch. Even lacking these features *Pariadens* would present a suitable morphotype from which later Cretaceous Stagodontidae might have arisen, if not for its relatively low trigonid and strongly developed entoconid, which we regard as autapomorphies.

The origin and dispersal of marsupials, in the context of plate tectonics, has been a subject of considerable interest^{15,26}. With several exceptions which represent isolated immigration events, fossil and/or living marsupials are restricted to North and South America, Antarctica and Australia. There are contrasting views on the continent from which marsupials originated that depend on varying interpretations of relationships within the order^{27,28}. Because of the close similarity of latest Cretaceous North and South American marsupial faunas, including identity at the generic level²⁹, it is believed that dispersal between North and South America occurred at that time³⁰. (Marsupials are, thus far, not known from pre-Maastrichtian South American mammal faunas; M. C. McKenna, personal communication). Based on present knowledge, the North American origins for the group would only require one, latest Cretaceous, immigration event. If marsupials originated on one of the southern continents, the presence of *Pariadens* in the Cenomanian of North America indicates that members of the group immigrated there much earlier than is generally supposed, and that postulation of a subsequent immigration event may be required to account for

the close similarity of latest Cretaceous North and South American marsupial faunas. Regardless, the presence of a specialized form in the Cenomanian indicates that marsupials were moderately diversified, at least, by that time.

We thank Gus Gustafson for leading us to the field area, Scott Madsen for preparing the specimen, the Biological Survey, New York State Museum and the Geology Department, Museum of Northern Arizona, for logistical support to R.L.C., and William Clemens, Jason Lillegraven and Larry Marshall for suggestions on the manuscript. Funding was provided by the University of Colorado Walker Van Riper Fund, and support and encouragement was provided by Malcolm McKenna.

Received 2 April; accepted October 30 1986.

- Lillegraven, J. A. *Paleontol. Contrib. Univ. Kansas* **50**, 1-122 (1969).
- Clemens, W. A., Lillegraven, J. A., Lindsay, E. H. & Simpson, G. G. in *Mesozoic Mammals: the First Two-Thirds of Mammalian History* (eds Lillegraven, J. A., Kielan-Jaworowska, Z. & Clemens, W. A.) 7-58 (University of California, Berkeley, 1979).
- Lillegraven, J. A. & McKenna, M. C. *Am. Mus. Novitates* **2840**, 1-68 (1986).
- Patterson, B. *Fieldiana: Geol.* **13**, 1-105 (1956).
- Kielan-Jaworowska, Z., Eaton, J. G. & Bown, T. in *Mesozoic Mammals: the First Two-Thirds of Mammalian History* (eds Lillegraven, J. A., Kielan-Jaworowska, Z. & Clemens, W. A.) 182-191 (University of California, Berkeley, 1979).
- Beliajeva, E. I., Trofimov, B. A. & Reshetov, V. J. in *Mesozoic and Cenozoic Faunas and Biostratigraphy of Mongolia* (eds Kramarenko, N. N. et al.) 19-45 (Joint Soviet-Mongolian Paleontological Expedition, Moscow, 1974).
- Nesov, L. A. *Vestnik Zoologii* **2**, 60-65 (1984).
- Fox, R. C. in *Early Mammals* (eds Kermack, D. M. & Kermack, K. A.); *Zool. J. Linn. Soc.* **50**, Suppl. 1, 145-164 (1971).
- Romans, R. C. *Pollen et Spores* **17**, 274-329 (1975).
- Cobban, W. A. & Hook, S. C. *Jurassic-Cretaceous Biochronology and Biogeography of North America* (ed. Westerman, G. E. G.) 257-271 (*Geol. Ass. Can. Spec. Pap.* **27**, 257-271 (1984)).
- Russell, L. S. *Geol. Ass. Can. Spec. Pap.* **13**, 137-161 (1975).
- Fox, R. C. *Geol. Ass. Can. Spec. Pap.* **18**, 577-594 (1978).
- Slaughter, B. H. *Science* **162**, 254-255 (1968).
- Turnbull, W. D. in *Dental Morphology and Evolution* (ed. Dahlberg, A. A.) 151-179 (University of Chicago Press, 1971).
- Tedford, R. H. in *Soc. econ. Paleontol. Mineral. Spec. Publ.* **21**, 109-126 (1974).
- Butler, P. M. *Breviora* **446**, 1-27 (1978).
- Clemens, W. A. in *Mesozoic Mammals: the First Two-Thirds of Mammalian History* (eds Lillegraven, J. A., Kielan-Jaworowska, Z. & Clemens, W. A.) 192-220 (Univ. California, Berkeley, 1979).
- Fox, R. C. *Can. J. Earth Sci.* **17**, 1489-1498 (1980).
- Lillegraven, J. A. *Evolution* **29**, 707-722 (1979).
- Butler, P. M. & Kielan-Jaworowska, Z. *Nature* **245**, 105-106 (1974).
- Kielan-Jaworowska, Z. *Palaeontol. Polonica* **33**, 103-132 (1975).
- Clemens, W. A. & Lillegraven, J. A. in *Vertebrates, Phylogeny and Philosophy* (eds Flanagan, K. & Lillegraven, J. A.) (University of Wyoming, 1986).
- Clemens, W. A. *Univ. California Publ. Geol. Sci.* **62**, 1-122 (1966).
- Marshall, L. G. in *Possums and Opossums, Studies in Evolution* (ed. Archer, M.) (Royal Zoological Society of New South Wales, in the press).
- Fox, R. C. *Can. J. Earth Sci.* **18**, 350-365 (1981).
- Marshall, L. G. in *Aspects of Vertebrate Evolution* (ed. Jacobs, L. L.) 345-386 (Museum of Northern Arizona Press, Flagstaff, 1980).
- Kirsch, J. A. in *Vertebrate Zoogeography & Evolution in Australasia* (eds Archer, M. & Clayton, G.) 627-632 (Hesperian, Victoria Park, 1984).
- Archer, M. in *Vertebrate Zoogeography & Evolution in Australasia* (eds Archer, M. & Clayton, G.) 633-807 (Hesperian, Victoria Park, 1984).
- Sigé, B. *Bull. Mus. nat. Hist. natur. ser. 3*, no. 99, sci. terr. 19, 375-405 (1972).
- Stehli, F. G. & Webb, S. D. *The Great American Interchange* (Plenum, New York, 1985).

Glutamate activates multiple single channel conductances in hippocampal neurons

C. E. Jahr & C. F. Stevens

Section of Molecular Neurobiology and the Howard Hughes Medical Institute, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510, USA

There is considerable evidence that glutamate is the principal neurotransmitter that mediates fast excitatory synaptic transmission in the vertebrate central nervous system¹⁻³. This single transmitter seems to activate two or three distinct types of receptors, defined by their affinities for three selective structural analogues of glutamate, NMDA (*N*-methyl-D-aspartate), quisqualate and kainate¹⁻⁶. All these agonists increase membrane permeability to monovalent cations⁷⁻⁹, but NMDA also activates a conductance that permits significant calcium influx^{10,11} and is blocked in a voltage-dependent manner by extracellular magnesium^{12,13}. Fast synaptic excitation seems to be mediated mainly by kainate/quisqualate receptors¹⁴⁻¹⁸, although NMDA receptors are sometimes activated¹⁹⁻²¹. We have investigated the properties of these conductances using single-channel recording²² in primary cultures of hippocampal neurons, because the hippocampus contains all subtypes of glutamate receptors^{23,24} and because long-term potentiation of synaptic transmission occurs in this structure^{25,26}. We find that four or more distinct single-channel currents are evoked by applying glutamate to each outside-out membrane patch. These conductances vary in their ionic permeability and in the agonist most effective in causing them to open. Clear transitions between all the conductance levels are observed. Our observations are compatible with the model that all the single channel conductances activated by glutamate reflect the operation of one or two complex molecular entities.

Figure 1a displays sample records obtained with prolonged application of 10 μ M glutamate, showing single channel currents with at least five distinct levels. The histogram (Fig. 1b) shows the relative frequencies of the various single channel currents. Small and large events are common but the medium-sized openings, although definitely present, are relatively infrequent. Chan-

nel openings in these three conductance categories are seen at all agonist concentrations (1 to 100 μ M), at all membrane potentials (-100 to +100 mV) tested, and during both brief and prolonged drug application. We have investigated particularly the events termed small (5-15 pS) and large (40-50 pS). The smallest events, with a conductance of about 5 pS, can be studied only at large holding potentials because they are too small, relative to equipment noise, for accurate measurements at many of the voltages used to study the large conductances. Medium-sized events (20-35 pS) are too infrequent for systematic investigation.

The selective glutamate agonists, NMDA, kainate and quisqualate, preferentially activate subpopulations of the conductances. NMDA tends to cause large openings, but kainate and quisqualate predominantly activate the small conductance (Fig. 1c), although all three agonists do produce some openings of all three sizes. The specific NMDA antagonist D-2-amino-5-phosphonovaleate (APV; ref. 1) prevents the activation of large events in response to NMDA, but does not eliminate the small openings (Fig. 1d). APV seems to have no effect on the small events produced by kainate and quisqualate; we do not know if APV prevents the infrequent openings of the large conductance induced by these two agonists. Quisqualate and kainate seem to activate the same conductance levels, although kainate preferentially produces events of conductance ≤ 5 pS whereas quisqualate activates 10 pS and 15 pS events more than kainate (Fig. 1c). Note that the distributions of events activated by all three agonists overlap: no agonist is specific for one conductance level.

The various conductance levels have different mean dwell times in the open state, and different ionic selectivities. The small conductance has a mean open time of ~ 0.5 ms, with no detectable dependence on membrane potential over the voltage range of -80 to +80 mV. This conductance is nearly equally permeable to sodium and caesium: its voltage-current relationship is linear and passes through the origin when the extracellular medium contains 165 mM sodium and the intracellular solution is 175 mM caesium. It does not permit detectable calcium ion permeation (Fig. 2b) and is not blocked by extracellular magnesium ions (Fig. 2d).

The large conductance (which varies from 40 to 50 pS from patch to patch) has two modes of gating: one has a short mean open time (1-3 ms); and the other, with a mean open time of 10-15 ms, occurs in bursts that can last several hundred milli-

Fig. 1 Glutamate activates single channel events of many different conductances. *a*, Examples of single channel events activated at -80 mV by glutamate ($10 \mu\text{M}$) recorded from an outside-out patch of membrane. *b*, Frequency histogram of the single channel conductances (no. of events, $n = 466$) activated by glutamate ($10 \mu\text{M}$) from a different patch than *a*. *c*, Histograms of data obtained from a single patch which was exposed serially to $10 \mu\text{M}$ NMDA ($n = 164$), $10 \mu\text{M}$ kainate ($n = 145$) and $2 \mu\text{M}$ quisqualate ($n = 202$) by the perfusion pipette method. *d*, Histograms from a different patch which was exposed to brief pressure applications of a solution containing $50 \mu\text{M}$ NMDA ($n = 125$) and then in a solution containing $50 \mu\text{M}$ NMDA and $200 \mu\text{M}$ APV ($n = 12$) although the concentrations at the patch are almost certainly lower through mixing with the superfusion medium. The effect of APV was reversible. The bin widths of all histograms is 2 pS.

Methods. Hippocampal neurons were obtained from the CA1 region of the hippocampus from 1–3-day-old newborn rats (Long-Evans) using the methods of Huettner and Baughman⁴¹. Small blocks of tissue ($<1 \text{ mm}^3$) were incubated in papain (20 units ml^{-1} ; Sigma or Cooper) for 1.5 h. The tissue was dissociated into a single cell suspension by trituration with a fire-polished Pasteur pipette and centrifuged through a balanced salt solution containing 50 mg ml^{-1} bovine serum albumin and 50 mg ml^{-1} trypsin inhibitor (both Sigma). The pellet was resuspended in complete growth medium MEM (Earle's salts, 20 mM glucose, 0.5 mM glutamine, 50 units ml^{-1} penicillin/streptomycin, 5% heat-inactivated rat serum) and plated onto glass coverslips coated with collagen/poly-D-lysine. Cultures were fed every 2–3 days by replacing half the volume of medium. Arabinosylcytosine ($5 \times 10^{-6} \text{ M}$) was added to cultures for 1 or 2 days during the first week after plating to suppress the proliferation of non-neuronal cells. All electrophysiology experiments were performed on outside-out patches of membrane from neurons grown for 2–4 weeks in culture. After an outside-out patch was obtained, the pipette tip was usually inserted into a perfusion pipette containing no agonist to insure that there were no spontaneous channel events. When spontaneous events were observed the patch was discarded. The external solution contained (in mM) NaCl 165; KCl 5, CaCl_2 2, HEPES 5 (pH adjusted to 7.3 with NaOH) unless otherwise noted. The internal solution contained (mM) CsCl 80, CsF 80, EGTA 10, HEPES 10 (pH adjusted to 7.3 with CsOH). Membrane current was filtered at 2,000 Hz or 4,000 Hz (-3 dB ; 8-pole Bessel) and digitally sampled at 100- μs or 50- μs intervals, respectively. For display purposes, records were sometimes digitally filtered by a Hanning filter routine. Chord conductances were calculated using 0 mV as the reversal potential (Fig. 2), as in normal concentrations of divalent cations the current-voltage relationships for all conductances were linear and reversed at the origin. Current levels were measured by fitting events with step functions and dwell times by measuring at half amplitude. Mean dwell times of the small conductances were computed as the mean of measured values. The distributions of the large conductance were fitted with exponentials and the mean open time estimated from the time constant (see Fig. 3). Drugs were applied to patches by pressure ejection⁴² from blunt pipettes (5–10 μm tip diameter) or by positioning the patch inside large diameter (30–50 μm) perfusion pipettes⁴³. Experiments were performed at room temperature (21–25 °C). Sources of chemicals: L-glutamic acid and kainic acid (Sigma); N-methyl-D-aspartic acid, quisqualic acid and D-2-amino-5-phosphonovaleic acid (Cambridge Research Biochemicals). Salts for recording solutions were obtained from Aldrich (Gold label) or Alfa (Puratronic).

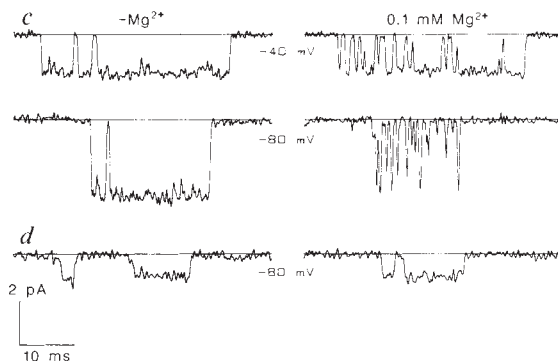
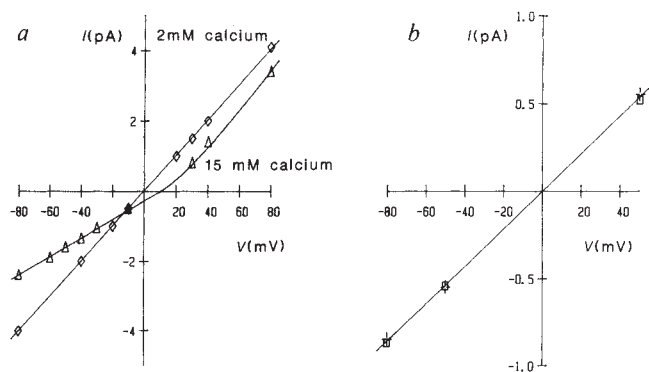
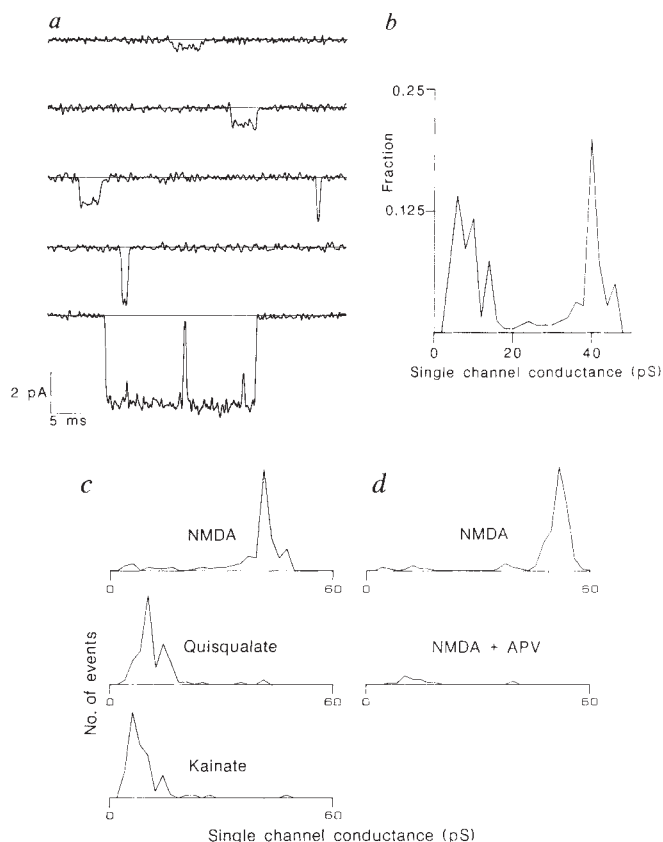


Fig. 2. The effects of external divalent cations on the large and small conductances. *a*, I - V curves of the large conductance generated by holding the patch at various potentials with $20 \mu\text{M}$ NMDA in standard recording solution containing 2 mM calcium, or in the same solution with 15 mM calcium. *b*, I - V curves of the 10-pS-conductance activated by quisqualate in a different patch than in *a* in recording solution containing either 2 or 15 mM calcium. *c*, Large conductance events activated by $20 \mu\text{M}$ NMDA at holding potentials of -40 and -80 mV in the absence of added magnesium and in the presence of 0.1 mM magnesium. *d*, From a different patch, small conductance events activated by $2 \mu\text{M}$ quisqualate in the presence and absence of external magnesium. Holding potential, -80 mV. Drugs were applied by perfusion pipettes.

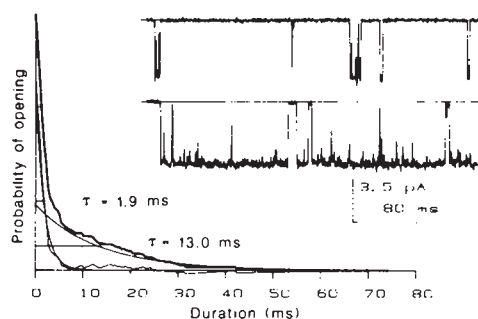


Fig. 3 The 50 pS conductance has two modes of gating. The single channel records illustrate (top) an example of the brief opening mode and (below) a burst of the long opening mode. Graph, the probability of openings plotted against their duration ($n = 256$). The uppermost curve (bold line) represents this relationship of all of the 50 pS events of a single patch activated by 20 μ M NMDA at a holding potential of -80 mV. An exponential curve (time constant, $\tau = 13$ ms) fitted by eye to the late portion of this curve was subtracted from it to give the rapidly decaying (lower) trace, which was fitted with another exponential curve ($\tau = 1.9$ ms).

seconds (Fig. 3). Mean open times were the same whether estimated as an average of the measured open duration or from exponentials fitted to the open time histogram (as in Fig. 3). The mean burst length varies greatly from patch to patch and can change with time in a single patch, suggesting that bursting may be modified by an unidentified regulatory mechanism. In both modes of gating, the large conductance has a significant permeability to calcium (see ref. 10) as well as to sodium and caesium, shown by a $+10$ mV shift in reversal potential when the extracellular concentration of calcium is increased from 2 to 15 mM (Fig. 2a). As Nowak *et al.*¹² reported, the large conductance level is blocked in a voltage-dependent manner by low micromolar concentrations of magnesium ions (Fig. 2c).

The histograms like those in Fig. 1 tend, with uniform experimental conditions, to be the same from patch to patch. This suggests that patches generally have the same relative numbers of small, medium and large conductance levels, even when only one channel of each size class appears in the records. The single channel conductance is typically used to define channel species. If two different conductance levels are observed in a patch, the usual conclusion is that the patch contains two different channel types representing distinct proteins; but this need not always be the case. For example, Labarca and Miller²⁷ and Krouse *et al.*²⁸ have reported chloride channels that appear to have distinct conductance levels (rather than a main level and a substate that is infrequently visited).

Is it possible that the glutamate receptor channel is one complicated molecular entity rather than a collection of independent and distinct molecular subtypes? We examined records from 126 patches for direct transitions between the three conductance levels (small, medium and large). If the three conductance levels were an expression of a single complex receptor/channel, we might expect to find occasional direct transitions between the various conductance states the molecule may occupy. Briefly, we have found transitions between all the conductance levels which cannot be attributed to simple superposition of independent events.

In 4 patches to which 10 μ M or 20 μ M glutamate had been applied, we observed 1,897 channel openings, 73 of which ($<4\%$) exhibited clear transitions from one level to another. A 'clear transition' is one that dwells for ≥ 0.3 ms at the state briefly visited, sufficient to be sure that the filter (8 pole Bessel, set at 2,000 Hz) response has settled. Transitions occur from each conductance level to every other one (Fig. 4). It is extremely unlikely that events with two conductance levels are due to the superimposition of openings of two or more independent channels. First, the frequencies of the transitions are much higher than predicted from the frequencies with which events of the component sizes occur from baseline. For instance, in the patch from which Fig. 4b was recorded, no 15 pS events were observed. Second, in many cases, events abruptly open to or close from the largest conductance. If these events were due to independent channels, the frequency with which their openings and closings would coincide should be vanishingly low. The transitions from the 50 pS to the 25–40 pS level are similarly affected by increased extracellular calcium. That is, at a given holding potential, high calcium decreases both the 50 pS and the lower conductances by the same percentage. This suggests that these transition states and the 50 pS conductance have similar permeability properties. Transitions from the large conductance to very small levels occur too infrequently to be analysed.

Glutamate receptors/channels do not appear to be identical in all regions of the CNS. In cultures of cerebellar granule neurons, glutamate activates a conductance very similar to the large events reported here²⁹. Much smaller conductances (in the femtosiemen range) were also reported but not channels in the 5–35 pS range²⁹. If the femtosiemen conductances are present in hippocampal neurons, they are too small for us to observe. Striatal neurons seem very similar to hippocampal neurons. Nowak, Ascher and colleagues report that NMDA activates the 50 pS channel which is blocked by magnesium¹² and permeable to calcium¹⁰; kainate predominately activates a 2 pS channel but also a 15–30 pS conductance, and quisqualate activates channels of ~ 8 pS³⁰. They report, however, that in the absence of magnesium the 50 pS conductance has a mean open time of ~ 5 ms and does not seem to exhibit prolonged bursts or subconductance states¹².

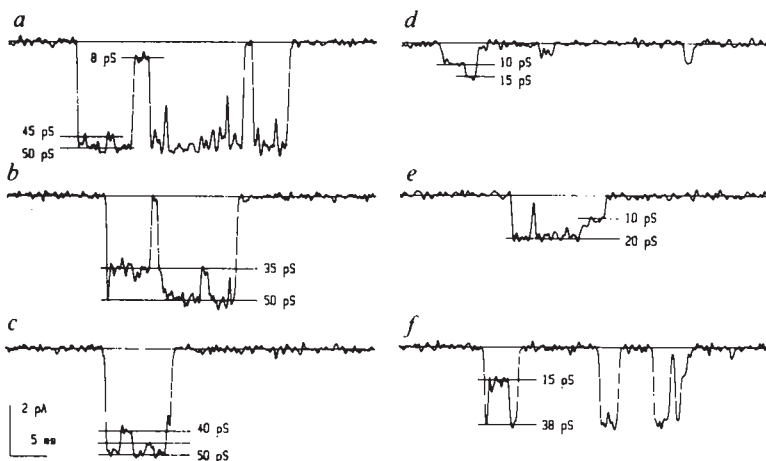


Fig. 4 Examples of transitions in conductance during single channel events taken from different patches. The agonist was either 10 μ M glutamate (d, e, f) or 20 μ M NMDA (a, b, c). All events were recorded at -80 mV.

A model that accounts for our data is a single receptor/channel complex with at least two separate binding sites, one with a higher affinity for NMDA and selectively blocked by APV, the other with a higher affinity for quisqualate and kainate; both sites bind glutamate. The model channel also needs several conformational states associated with different conductance levels and, for at least some levels, different permeability properties. The probability of occupancy of each level would be determined in part by which binding site is occupied. Preliminary data suggests that glutamate concentration is one determinant of the conductance level, with low concentrations favouring the large level. Differential binding of the selective agonists in various regions of the CNS^{31,32} could be explained by differential expression of receptor subunits or by differences in post-translational modification of the complex. This hypothesis is unattractive as it assigns greater complexity to a single molecular complex than previously recognized, but it does have the advantage that fewer channel types need to be present in each patch. If different subtypes of glutamate receptor channels are to function together, combining them into a single entity would assure that the same balance of subtypes is always present. Another model is that two or three distinct channels exist in all our patches and that one or more of these channels has a major conductance and several substates with conductances that happen to coincide with the values for the other channels.

Whether all of the conductance levels represent a single membrane protein complex, or whether each conductance level corresponds to a distinct channel, the membrane receptor/channels subserving excitatory amino-acid synaptic transmission in the CNS provide a far richer range of functional attributes than expected from studies of the nicotinic acetylcholine receptor^{10,12,29,30}. The more complex properties of excitatory receptors in the CNS may provide flexibility for neuronal information transfer and storage.

The complexity may be particularly important in long-term potentiation (LTP). LTP is an increase in efficacy of excitatory transmission that occurs when a synapse is repetitively activated and the postsynaptic neuron is sufficiently depolarized^{25,26,33-35}. This is thought to activate NMDA receptors by removing the voltage-dependent magnesium block^{12,13}. When the NMDA-activated large conductance is open, calcium ions enter and the local intracellular calcium concentration increases (see also refs 10 and 11). This may activate calcium-dependent enzymes such as type II Ca/calmodulin-dependent protein kinase (see refs 36-40 for discussion). Fast excitatory transmission in the hippocampus, on the other hand, is mediated mainly by the kainate/quisqualate-activated conductances, which are not blocked by magnesium and are not permeable to calcium; they do not induce LTP.

Although we have elucidated some of the complexities of glutamate excitation of hippocampal neurons by studying single channel properties, we have not yet been able to understand fully all of the variables that determine the preferential use of one conductance level or another. Future work on this system should reveal further the subtleties of excitatory synaptic transmission required for the operation of the brain.

This work was supported by NIH grants NS21419-02(3) (C.E.J.) and NS12961-11(16) and The Howard Hughes Medical Institute (C.F.S.).

Received 6 August; accepted 2 December 1986.

- Watkins, J. C. & Evans, R. H. *A. Rev. Pharmacol. Tox.* **21**, 165-204 (1981).
- McLennan, H. in *Glutamate as a Neurotransmitter* (eds Di Chiara, G. & Gessa, G. L.) 253-262 (Raven, New York, 1981).
- Foster, A. C. & Fagg, G. E. *Brain Res. Rev.* **7**, 103-164 (1984).
- O'Brien, R. J. & Fischbach, G. D. *J. Neurosci.* **6**, 3275-3283 (1986).
- Ishida, A. T. & Neyton, J. *Proc. natn. Acad. Sci. U.S.A.* **82**, 1837-1841 (1985).
- Kiskin, N. I., Krishtal, O. A. & Tsyndrenko, A. Ya. *Neurosci. Lett.* **63**, 225-230 (1986).
- MacDonald, J. F. & Wojtowicz, J. M. *Can. J. Physiol. Pharmac.* **58**, 1393-1397 (1980).
- Crunelli, V., Forda, S. & Kelly, J. S. *J. Physiol., Lond.* **351**, 327-342 (1984).
- Habitz, J. J. & Langmoen, I. A. *J. Physiol. Lond.* **325**, 317-331 (1982).
- Ascher, P. & Nowak, L. *J. Physiol., Lond.* **377**, 43P (1986).

- MacDermott, A. B., Mayer, M. L., Westbrook, G. L., Smith, S. J. & Barker, J. L. *Nature* **321**, 519-522 (1986).
- Nowak, L., Bregestovski, P., Ascher, P., Herbet, A. & Prochiantz, A. *Nature* **307**, 462-465 (1984).
- Mayer, M. L., Westbrook, G. L. & Guthrie, P. B. *Nature* **309**, 261-263 (1984).
- Collingridge, G. L., Kehl, S. J. & McLennan, H. *J. Physiol., Lond.* **334**, 19-31 (1983).
- Crunelli, V., Forda, S. & Kelly, J. S. *J. Physiol., Lond.* **341**, 627-640 (1983).
- Jahr, C. E. & Jessell, T. M. *J. Neurosci.* **5**, 2281-2289 (1985).
- Jahr, C. E. & Yoshioka, K. *J. Physiol., Lond.* **370**, 515-530 (1986).
- Nelson, P. G., Pun, R. Y. K. & Westbrook, G. L. *J. Physiol., Lond.* **372**, 169-190 (1986).
- Thomson, A. M. *J. Physiol., Lond.* **370**, 531-549 (1986).
- Coan, E. J. & Collingridge, G. L. *Neurosci. Lett.* **53**, 21-26 (1985).
- Dale, N. & Roberts, A. *J. Physiol., Lond.* **363**, 35-59 (1985).
- Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Pflügers Arch. ges. Physiol.* **391**, 85-100 (1981).
- Habitz, J. J. *Brain Res.* **247**, 149-153 (1982).
- Crunelli, V., Forda, S. & Kelly, J. S. *J. Physiol., Lond.* **351**, 327-342 (1984).
- Bliss, T. V. P. & Lomo, T. J. *J. Physiol., Lond.* **232**, 331-356 (1973).
- Bliss, T. V. P. & Gardner-Medwin, A. R. *J. Physiol. Lond.* **232**, 357-374 (1973).
- Labarca, P. & Miller, C. J. *Membrane Biol.* **61**, 31-38 (1981).
- Krouse, M. E., Schneider, G. T. & Gage, P. W. *Nature* **319**, 58-60 (1986).
- Cull-Candy, S. G. & Ogden, D. C. *Proc. R. Soc. B224*, 367-373 (1985).
- Ascher, P. & Nowak, L. *Proc. int. Un. physiol. Sci.* **16**, 382 (1986).
- Greenamyre, J. T., Olson, J. M. M., Penney, J. B. & Young, A. B. *J. Pharmac. Exp. Ther.* **233**, 254-263 (1985).
- Monaghan, D. T., Holets, V. R., Toy, D. W. & Cotman, C. W. *Nature* **306**, 176-179 (1983).
- Wigstrom, H., Gustafsson, B., Huang, Y. Y. & Abraham, W. C. *Acta physiol. scand.* **126**, 317-319 (1986).
- Malinow, R. & Miller, J. P. *Nature* **320**, 529-530 (1986).
- Kelso, S. R., Ganong, A. H. & Brown, T. H. *Proc. natn. Acad. Sci. U.S.A.* **83**, 5326-5330 (1986).
- Kennedy, M. B., Bennett, M. K. & Erondy, N. E. *Proc. natn. Acad. Sci. U.S.A.* **80**, 7357-7361 (1983).
- Ouimet, C. C., McGuinness, T. L. & Greengard, P. *Proc. natn. Acad. Sci. U.S.A.* **81**, 5604-5608 (1984).
- Miller, S. G. & Kennedy, M. B. *Cell* **44**, 861-870 (1986).
- Saitoh, T. & Schwartz, J. H. *J. Cell Biol.* **100**, 835-842 (1985).
- Lai, Y., Nairn, A. C. & Greengard, P. *Proc. natn. Acad. Sci. U.S.A.* **83**, 4253-4257 (1986).
- Huettnner, J. E. & Baughman, R. W. *J. Neurosci.* **6**, 3044-3061 (1986).
- Choi, D. W. & Fischbach, G. D. *J. Neurophysiol.* **45**, 605-620 (1981).
- Yellen, G. *Nature* **296**, 357-359 (1982).

Multiple-conductance channels activated by excitatory amino acids in cerebellar neurons

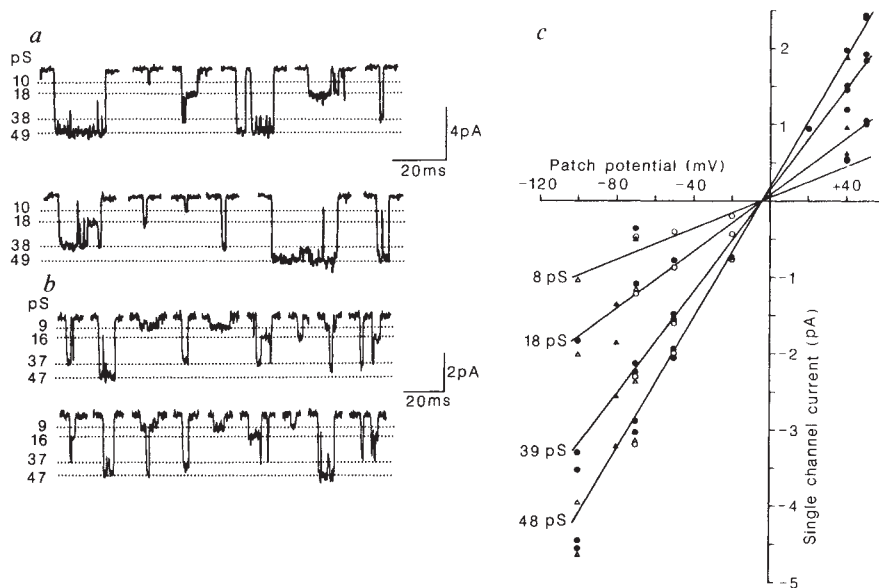
Stuart G. Cull-Candy & Maria M. Usowicz

MRC Receptor Mechanisms Research Group, Department of Pharmacology, University College London, Gower Street, London WC1E 6BT, UK

In the mammalian central nervous system amino acids such as L-glutamate and L-aspartate are thought to act as fast synaptic transmitters^{1,2}. It has been suggested that at least three pharmacologically-distinguishable types of glutamate receptor occur in central neurons and that these are selectively activated by the glutamate analogues N-methyl-D-aspartate (NMDA), quisqualate and kainate³⁻⁵. These three receptor types would be expected to open ion channels with different conductances. Hence if agonists produce similar channel conductances this would suggest they are acting on the same receptor^{6,7}. Another possibility is suggested by experiments on spinal neurons⁸, where GABA (γ -amino butyric acid) and glycine appear to open different sub-conductance levels of one class of channel while acting on different receptors. By analogy, several types of glutamate receptor could also be linked to a single type of channel with several sub-conductance states. We have examined these possibilities in cerebellar neurons by analysing the single-channel currents⁹ activated by L-glutamate, L-aspartate, NMDA, quisqualate and kainate in excised membrane patches. All of these agonists are capable of opening channels with at least five different conductance levels, the largest being about 45-50 pS. NMDA predominantly activated conductance levels above 30 pS while quisqualate and kainate mainly activated ones below 20 pS. The presence of clear transitions between levels favours the idea that the five main levels are all sub-states of the same type of channel.

Single-channel currents were recorded in outside-out patches from large cerebellar neurons (soma diameters >30 μ m). Care

Fig. 1 Single-channel currents in two outside-out membrane patches (*a*) activated by $10\ \mu\text{M}$ aspartate ($V_m = -100\ \text{mV}$), and (*b*), activated by $10\ \mu\text{M}$ quisqualate ($V_m = -70\ \text{mV}$). The single-channel currents are composed of four discrete levels indicated by dotted lines. The slope conductances for aspartate channels are 49, 38, 18 and $10\ \text{pS}$, and for quisqualate channels are 47, 37, 16 and $9\ \text{pS}$. Calibration: $4\ \text{pA}$, $2\ \text{pA}$ and $20\ \text{ms}$; for illustrations the records were low-pass filtered at $2\ \text{kHz}$ ($-3\ \text{dB}$). *c*, Relationship between single-channel current amplitude and patch-potential for channels activated by: $\bullet$, NMDA (10 and $50\ \mu\text{M}$); Δ , aspartate ($10\ \mu\text{M}$); $\blacktriangle$, glutamate ($10\ \mu\text{M}$); and $\circ$, quisqualate ($10\ \mu\text{M}$) in four different membrane patches. Least-squares lines yielded slope conductances of 48, 39, 18 and $8\ \text{pS}$. Reversal potential for channels activated by these four agonists was close to $-5\ \text{mV}$ and fitted lines were constrained to pass through this value. Note the presence of a current level activated by glutamate at $-80\ \text{mV}$ and by NMDA at $+40\ \text{mV}$ between the 18 and $39\ \text{pS}$ conductance levels. Records were digitized and individual currents fitted by the response function of the recording system to a step input²⁷. Mean current levels were determined from maximum-likelihood fits of sums of gaussian distributions to cursor-fitted amplitudes²⁷, and were plotted against membrane potential to yield slope conductances. The composition of the bathing solution facing the extracellular side of the patch was, in mM : $150\ \text{NaCl}$, $2.8\ \text{KCl}$, $1\ \text{CaCl}_2$ and $10\ \text{Na-HEPES}$, $\text{pH}\ 7.2$. The pipette solution facing the intracellular side was in mM : $140\ \text{CsCl}$, $4\ \text{NaCl}$, $0.5\ \text{CaCl}_2$, 5 potassium EGTA and $10\ \text{K-HEPES}$, $\text{pH}\ 7.2$. Drugs were applied in the bathing medium through a plastic tube placed beneath the water immersion objective. This gave responses which developed in less than $5\ \text{s}$. The bathing solution was thoroughly exchanged between agonist applications to avoid the possibility of residual drug in the bath. Solutions were Mg^{2+} -free^{15,26}. Experiments were carried out at room temperature (20 – 22°C). Cerebellar neurons were cultured on poly-L-lysine-coated coverslips for 6–14 days as previously described¹⁴ in Dulbecco's modified Eagle's medium, supplemented with 10% fetal calf serum and 10% horse serum. Four days after plating, cells were treated for $48\ \text{h}$ with cytosine arabinoside ($10^{-5}\ \text{M}$) to inhibit non-neuronal cells and dividing neurons, including granule cells which are the predominant neurons in untreated cultures²⁸. Granule cells were virtually absent or occurred at low density in these cultures. The characteristic size ($<10\ \mu\text{m}$), capacitance, electrophysiological and morphological characteristics of granule cells allowed them to be readily distinguished from the larger neurons, presumed to be Purkinje cells, which were used in this study.



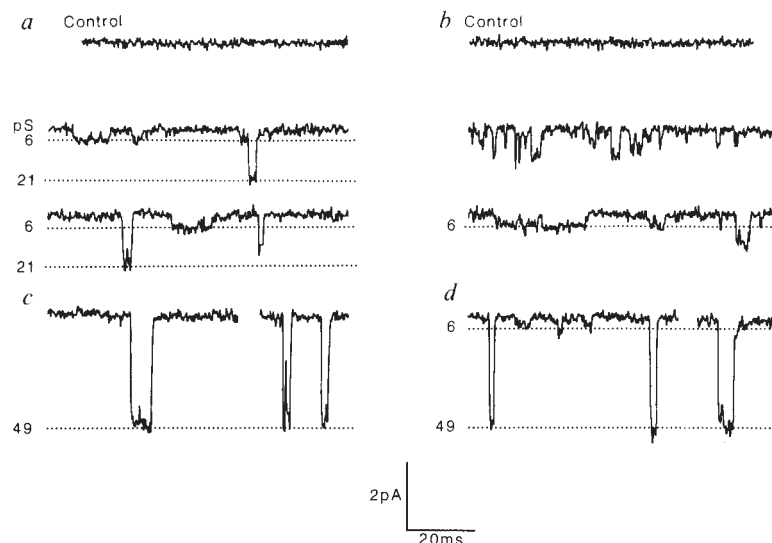
was taken to avoid granule cells which were occasionally seen and can be readily identified under Nomarski optics (Fig. 1, legend). Neurons from fetal rats (17–19 days old) were grown in tissue culture with methods established for maintaining Purkinje cells *in vitro*^{10–13}. Purkinje cells from young rats respond to quisqualate and NMDA although NMDA sensitivity is low in the adult neurons¹⁴. Glutamate, aspartate, NMDA and quisqualate all activate channels with a maximum conductance of about $50\ \text{pS}$ as well as lower conductance openings. A total of four or five conductance levels can be detected which appear similar for all agonists. The recordings in Fig. 1*a* and *b* are examples of single-channel currents activated by $10\ \mu\text{M}$ aspartate ($-100\ \text{mV}$) and $10\ \mu\text{M}$ quisqualate ($-70\ \text{mV}$) in two outside-out patches. The levels of four distinct conductances are indicated by dotted lines. Similar records were obtained with 3 – $10\ \mu\text{M}$ glutamate and 10 – $50\ \mu\text{M}$ NMDA (data not shown).

The dependence of current amplitude on patch-potential is plotted in Fig. 1*c* for channels activated by glutamate, aspartate, NMDA and quisqualate. Interpolation gives an estimated reversal potential of $-3.8 \pm 0.4\ \text{mV}$ (mean $\pm$ s.e.m., 9 patches). The mean values for the five main conductance levels were: $48 \pm 0.7\ \text{pS}$ (mean $\pm$ s.e.m., 23 experiments); $38 \pm 0.8\ \text{pS}$ (12 experiments); $28 \pm 1.4\ \text{pS}$ (7 experiments); $18 \pm 0.5\ \text{pS}$ (13 experiments) and $8.3 \pm 0.6\ \text{pS}$ (17 experiments). These estimates were obtained by pooling slope conductances and chord conductances obtained with all agonists. The largest current level probably corresponds to the previously reported $50\ \text{pS}$ channel^{15,16}, activated by glutamate and NMDA. Patches varied in their responses and 5 levels were not always detected. The $28\ \text{pS}$ level occurred infrequently. At least 3 or 4 levels were activated by glutamate (3 – $10\ \mu\text{M}$) in 18 out of 25 patches, by aspartate (3 – $10\ \mu\text{M}$) in 14 out of 16 patches, by NMDA (10 – $50\ \mu\text{M}$) in 18 out of 20 patches and by quisqualate (10 – $50\ \mu\text{M}$) in 5 out of 26 patches.

Kainate can activate similar and also smaller conductances. Figure 2 shows currents activated by $10\ \mu\text{M}$ and $50\ \mu\text{M}$ kainate, and $10\ \mu\text{M}$ NMDA in a single outside-out patch. Kainate ($10\ \mu\text{M}$) activated conductance levels of $21\ \text{pS}$ and $6\ \text{pS}$ (Fig. 2*a*) which were similar to the two smallest levels detected with other agonists. When the kainate concentration was increased to $50\ \mu\text{M}$, openings of $49\ \text{pS}$ were also obtained (Fig. 2*d*). These are very similar in size to the largest currents produced by $10\ \mu\text{M}$ NMDA in the same patch (Fig. 2*c*). The increased opening frequency produced by $50\ \mu\text{M}$ kainate was accompanied by 'noisy' currents (Fig. 2*b*) which appeared less discrete and did not correspond to the levels produced by $10\ \mu\text{M}$ kainate. Indeed, in 8 out of 10 outside-out patches no discrete single-channel currents were evoked by kainate, but only an inward current and a noise increase. Analysis of this noise^{17,18} gave estimates of the apparent single-channel conductance of $1.0 \pm 1.5\ \text{pS}$ ($\pm$ s.e.m., 8 patches) for $10\ \mu\text{M}$ kainate, and $1.8 \pm 0.5\ \text{pS}$ (7 patches) for $50\ \mu\text{M}$ kainate. These values, which are expected to reflect a weighted average of the actual single-channel conductances (if each state has a low occupancy), are smaller than those shown in Figs 1 and 2. This suggests that kainate activates additional conductance levels that are too small to be resolved as discrete events. These values are similar to those reported previously for kainate channels in embryonic mouse neurons¹⁹ and retinal horizontal cells²⁰. Quisqualate was the only other agonist examined which produced noise increases (as well as 6 and $21\ \text{pS}$ channel openings). Kainate and quisqualate also activate at least three conductances, including the maximum level of about $50\ \text{pS}$, in mouse midbrain neurons¹⁹.

The data presented so far indicate that NMDA, quisqualate and kainate are all capable of activating the $50\ \text{pS}$ conductance level as well as the same lower conductance levels. But they do not do so with equal probabilities: Fig. 3*a* and *b* shows amplitude histograms of currents activated by $3\ \mu\text{M}$ glutamate and

Fig. 2 Single-channel currents activated by kainate and NMDA in the same patch. Control traces (in the absence of agonist) are shown at the top of each set of records. Outside-out patch held at -70 mV: *a*, $10 \mu\text{M}$ kainate activates two discrete conductance levels of 21 and 6 pS (values calculated for the mean reversal potential of -3.8 mV); *b*, $50 \mu\text{M}$ kainate activates a higher frequency of openings. Amplitudes are more variable and not all correspond to those activated by $10 \mu\text{M}$ kainate. *c*, 49 pS channel-currents activated in this patch by $10 \mu\text{M}$ NMDA. *d*, Selected larger currents (49 pS) activated in the same patch by $50 \mu\text{M}$ kainate. These higher conductance levels were not seen in the presence of $10 \mu\text{M}$ kainate. The 49 pS openings produced by $10 \mu\text{M}$ NMDA and by $50 \mu\text{M}$ kainate could be due to the activation of the same receptors. Alternatively, the overall increase in opening frequency, at high kainate concentrations, may result in an increased probability of detection of 49 pS openings. Calibration 2 pA and 20 ms; records low-pass filtered at 1 kHz (-3 dB).



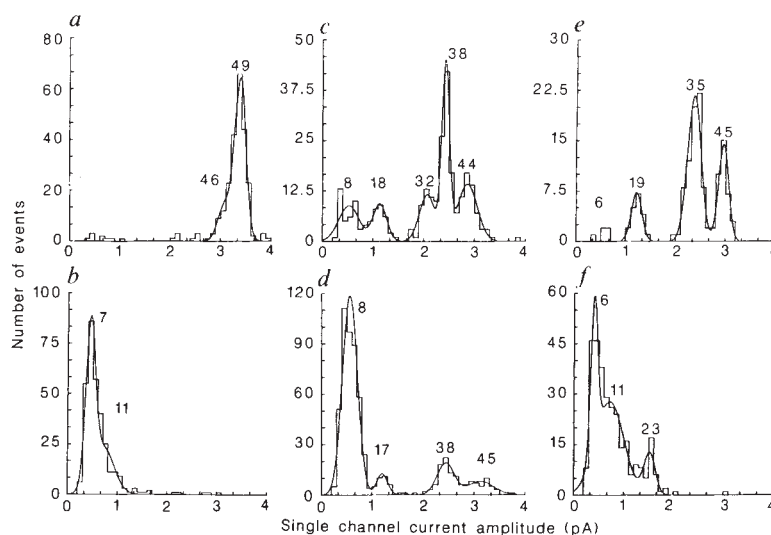
$10 \mu\text{M}$ quisqualate applied to a single patch held at -70 mV. Glutamate activated mainly 49 pS openings whereas quisqualate activated mainly 7 pS openings. Both histograms, however, show a second component adjacent to the main one. The amplitude histograms in Fig. 3*c* and *d* are from a patch exposed to $10 \mu\text{M}$ aspartate and $10 \mu\text{M}$ quisqualate. The histogram for aspartate currents is fitted with five gaussian distributions with the largest peaks above 30 pS. In contrast quisqualate openings display four amplitude levels with the majority occurring below 20 pS (quisqualate currents in Fig. 1 are from this patch). Fig. 3*e* and *f* shows amplitude histograms for NMDA and kainate currents in different patches. Typically, amplitude histograms for NMDA consisted of a majority of conductances above 30 pS, whereas kainate activated levels mainly below 20 pS.

Measurements of amplitude distributions indicate that the fraction of openings with conductances above 30 pS was 0.8 ± 0.02 (mean $\pm$ s.e.m., 13 experiments) for glutamate, aspartate and NMDA (3 – $50 \mu\text{M}$). This compares with 0.27 ± 0.08 (7 experiments) for quisqualate (3 – $50 \mu\text{M}$), and less than 0.1 for kainate ($50 \mu\text{M}$). The values were derived from the relative areas

under the gaussian distributions of the amplitude histograms (Fig. 3, legend). Clearly, the agonists preferentially activated different conductances but none was specific for one level. In addition, the mean lifetime of individual 45–50 pS openings (estimated as the time constant of their distributions) was usually longer than that of the lower conductance levels. The 45–50 pS mean lifetimes produced by glutamate, aspartate, NMDA (3 – $50 \mu\text{M}$) and $50 \mu\text{M}$ quisqualate were in the range 5.2–7.1 ms at -70 mV. However, $10 \mu\text{M}$ quisqualate gave a mean lifetime of 2.2 ms. This compares with a mean lifetime of 0.7–2.0 ms for the lower conductance levels when opened by all the agonists. The exception was $50 \mu\text{M}$ NMDA which gave longer duration events (2.7 ms for 38 pS openings and 5.2 ms for 18 pS openings).

The various conductance levels produced by the amino-acid analogues could result from the activation of channels with different conductances, or from the presence of different sub-conductance states of a single channel type. The second alternative is supported by the clear transitions between levels which are apparent in Figs 1 and 4. In different patches 2–20% of all openings were followed by transitions to another open level,

Fig. 3 Distributions of amplitudes of single-channel currents, illustrating the relative proportions of the current levels activated by different amino acids. Estimates of conductances are given above each peak. *a*, *b*, Channels activated by (a) $3 \mu\text{M}$ glutamate, and (b) $10 \mu\text{M}$ quisqualate, when applied to the same outside-out patch ($V_m = -70$ mV for all histograms). Glutamate ($3 \mu\text{M}$) produced a main conductance level of 49 pS whereas quisqualate produced predominantly 7 pS openings. Both also gave a second level adjacent to main peak. *c*, *d*, Channels activated by (c) $10 \mu\text{M}$ aspartate, and (d) $10 \mu\text{M}$ quisqualate, applied to a single outside-out patch (different patch from *a*, *b*). Aspartate gave five conductance levels with a majority above 30 pS. Quisqualate gave four levels in this patch (contrast with *b*); conductances below 20 pS were preferentially activated. *e*, Channels in the presence of $10 \mu\text{M}$ NMDA (applied to a different patch). NMDA opened predominantly conductances above 30 pS, although a lower level is present. *f*, Channels in the presence of $10 \mu\text{M}$ kainate, which mainly gave conductances below 20 pS. Mean current levels were taken from the simultaneous fit of several Gaussian components²⁷ (shown as continuous lines) to the cursor-fitted amplitudes. Mean currents were converted either to slope conductances (from the current-voltage relationship of each patch) or to chord conductances, employing the mean reversal potential of -3.8 mV. The mean lifetime of 44–49 pS openings was about 6 ms for glutamate (3 – $10 \mu\text{M}$) and about 2 ms for quisqualate (10 or $20 \mu\text{M}$). The resolution for detection of openings was 60–150 μs but openings had to be at least 0.5 ms for their amplitude to be measurable; correction for omission of 44–49 pS openings shorter than 0.5 ms does not substantially alter our conclusions.



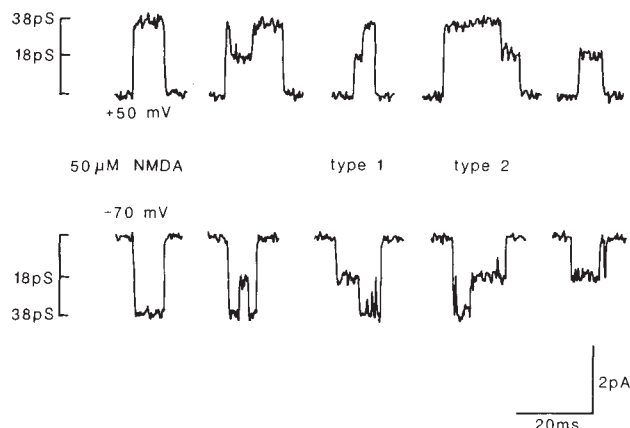


Fig. 4 Examples of the various forms of transition that occurred between the 38 pS and 18 pS conductance levels. Examples of a single opening to the 38 pS level and the 18 pS level are also included. These were selected from openings activated by 50 μ M NMDA at (top) +50 mV and (below) -70 mV. Of events containing a single transition between these two levels (labelled type 1 and type 2) 75% were type 2 (step from 38 pS to 18 pS) at -70 mV and 80% were type 2 at +50 mV. Calibration: 2 pA and 20 ms, 1 kHz filtering, -3 dB. In this patch 214 openings of 38 pS and 41 openings of 18 pS occurred in isolation in a 16.4 s period; 306 openings of 38 pS were directly associated with 244 openings of 18 pS, and a smaller number of these openings were also associated with other levels. The 38 pS openings occurring in isolation opened directly from, and closed directly to, the baseline with no detectable inflection on the rising or falling phases. If each of these were due to the superimposition of 18 pS openings, then two 18 pS openings would have opened and shut simultaneously on 214 occasions. The probability of this occurring is extremely low, especially as there were relatively few isolated 18 pS events. A similar argument applies to the possibility that separate 18 pS and 38 pS openings were so close that no gap could be detected between them.

rather than shutting. Transitions between 18 and 8 pS levels are not shown (but were readily identified, especially in response to quisqualate). We observed no direct transitions between 48 pS and 18 pS levels, although they could be associated indirectly via the 38 pS sub-level within single events. The presence of these direct transitions favours the idea that the levels are sub-states of the same type of channel. However, it is unclear whether the noise increase which was sometimes produced by kainate and quisqualate was also associated with the same channel.

Events containing transitions between 38 and 18 pS were analysed in more detail because the large size of this transition made it easy to identify. Figure 4 shows examples of the 38 and 18 pS openings in isolation and several forms of direct transition between them in a patch held at -70 and +50 mV in the presence of 50 μ M NMDA. Steps from 18 pS to 38 pS were much rarer than steps from 38 pS to 18 pS. Furthermore, reversing the direction of current flow through the channel did not appreciably influence this asymmetry (Fig. 4). The asymmetry, which may occur for other types of receptor-channel^{21,22} suggests that the channel mechanism does not obey microscopic reversibility. Its lack of voltage dependence suggests that this is not a result of coupling of ion flow to the gating mechanism²³. The asymmetry was independent of agonist. For example, of all events containing one transition (type 1 and type 2 in Fig. 4) $77 \pm 2.4\%$ (mean $\pm$ s.e.m., 10 experiments) were of type 2 (step from 38 pS to 18 pS) for glutamate, aspartate and NMDA (10–50 μ M), and $69 \pm 4\%$ (3 experiments) were type 2 for quisqualate (10 μ M). The asymmetry and its lack of dependence on agonist suggest that these two levels are sub-states of the same channel and do not represent separate channels. This is also supported by the absence of a detectable inflection on the rising and falling phases of

events, which would be present if 38 pS currents were composed of superimposed 18 pS openings (Fig. 4, legend).

Recent experiments have demonstrated a Ca^{2+} permeability of the conductance mechanism linked to NMDA receptors, with little or no Ca^{2+} permeability of the conductance mechanism associated with kainate receptors^{24,25}. Furthermore, channels opened by NMDA are blocked by Mg^{2+} (refs 15, 26). We have found that whole-cell currents activated by 50 μ M aspartate (-70 mV) were almost completely blocked in the presence of 1 mM Mg^{2+} . In the same cell the apparent conductance of quisqualate-activated channels, estimated from noise analysis, was reduced to roughly one half by Mg^{2+} while the apparent conductance of channels opened by kainate (20 μ M) was virtually unchanged. This is consistent with the idea that Mg^{2+} selectively blocks the large conductance openings and hence that the ionic selectivity of the excitatory amino-acid channels varies with substrate, in contrast with anion flux through GABA and glycine channels⁸.

Our experiments exclude the idea that the three proposed types of glutamate receptor are coupled to separate channels with different unitary conductances. Previous results with antagonists make it unlikely that there is only one type of glutamate receptor, the mode of activation being dependent on the agonist used. It seems more likely that there is one class of channel which can adopt any of a number of major conductance levels. This could be simultaneously linked to several different receptor types, or alternatively each channel could be coupled to a single receptor. In either case the predominant conductance level would depend on the receptor type that was activated and either possibility is compatible with the existence of regional differences in the proportions of kainate, quisqualate and NMDA binding sites in the CNS⁴. If each channel is coupled to only one receptor, the sharing of similar conductance states by the channels makes it likely that the channel protein molecule will be similar in each type of receptor-channel complex; indeed each type of complex could be a variation on a common structural theme.

This work was supported by the MRC and the Wellcome Trust. We thank David Colquhoun for valuable discussion, Paula Dilger for expert help with tissue culture and J. C. Watkins for a gift of NMDA. S.G.C.-C. is a Wellcome Senior Lecturer and M.M.U. is supported by the MRC.

Received 12 September; accepted 27 November 1986.

1. Curtis, D. R. & Johnston, G. A. R. *Ergebn. Physiol.* **69**, 97–188 (1974).
2. Krnjevic, K. *Physiol. Rev.* **54**, 418–540 (1974).
3. Watkins, J. C. & Evans, R. H. A. *Rev. Pharmac. Toxicol.* **21**, 165–204 (1981).
4. Foster, A. C. & Fagg, G. E. *Brain Res. Rev.* **7**, 103–164 (1984).
5. Mayer, M. L. & Westbrook, G. L. *J. Physiol., Lond.* **384**, 29–53 (1984).
6. Cull-Candy, S. G., Miledi, R. & Parker, I. *J. Physiol., Lond.* **321**, 195–210 (1981).
7. Gardner, P., Ogden, D. C. & Colquhoun, D. *Nature* **309**, 160–162 (1984).
8. Hamill, O. P., Bormann, J. & Sakmann, B. *Nature* **305**, 805–808 (1983).
9. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Pflügers Arch. ges. Physiol.* **391**, 85–100 (1981).
10. Moonen, G., Neale, E. A., Macdonald, R. L., Warren, G. & Nelson, P. G. *Dev. Brain Res.* **5**, 59–73 (1982).
11. Weber, A. & Schachner, M. *Brain Res.* **311**, 119–130 (1984).
12. Gruol, D. L. *Brain Res.* **263**, 223–241 (1983).
13. Cull-Candy, S. G. & Usowicz, M. M. *Brain Res.* (in the press).
14. Crepel, F., Dupont, J.-L. & Gardette, R. *Brain Res.* **279**, 311–315 (1983).
15. Nowak, L., Bregestovski, P., Ascher, P., Herbet, A. & Prochiantz, A. *Nature* **307**, 462–465 (1984).
16. Cull-Candy, S. G. & Ogden, D. C. *Proc. R. Soc. B* **224**, 367–373 (1985).
17. Katz, B. & Miledi, R. *J. Physiol., Lond.* **224**, 665–699 (1972).
18. Anderson, C. R. & Stevens, C. F. *J. Physiol., Lond.* **235**, 655–691 (1973).
19. Ascher, P., Nowak, L. & Kehoe, J. S. in *Ion Channels in Neural Membranes* (eds Ritchie, J. M., Keynes, R. D. & Bolis, L.) 823–295 (Liss, New York, 1986).
20. Ishida, A. T. & Neyton, J. *Proc. natn. Acad. Sci. U.S.A.* **82**, 1837–1841 (1985).
21. Hamill, O. P. & Sakmann, B. *Nature* **294**, 462–464 (1981).
22. Trautmann, A. *Nature* **298**, 272–275 (1982).
23. Lüscher, P. *Biophys. J.* **47**, 581–590 (1985).
24. MacDermott, A. B., Mayer, M. L., Westbrook, G. L., Smith, S. J. & Barker, J. L. *Nature* **321**, 519–522 (1986).
25. Ascher, P. & Nowak, L. *J. Physiol., Lond.* **377**, 35P (1986).
26. Mayer, M. L., Westbrook, G. L. & Guthrie, P. B. *Nature* **309**, 261–263 (1984).
27. Colquhoun, D. & Sigworth, F. J. in *Single Channel Recording* (eds Sakmann, B. & Neher, E.) 191–264 (Plenum, New York, 1983).
28. Cull-Candy, S. G. & Ogden, D. C. in *Ion Channels in Neural Membranes* (eds Ritchie, J. M., Keynes, R. D. & Bolis, L.) 297–308 (Liss, New York, 1986).

Glycine potentiates the NMDA response in cultured mouse brain neurons

J. W. Johnson & P. Ascher

Laboratoire de Neurobiologie, Ecole Normale Supérieure,
46 rue d'Ulm, 75005 Paris, France

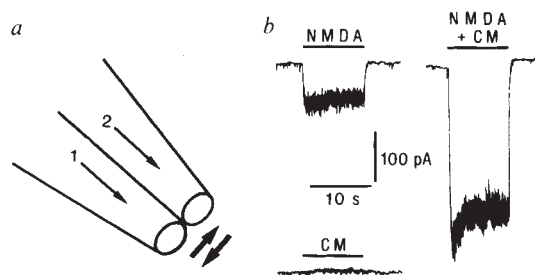


Fig. 1 Effect of conditioned medium on the response to NMDA. Whole cell recording. Internal solution, CsCl. Holding potential, -50 mV. Agonists were applied using the system illustrated in *a* (and see methods). *b*, NMDA ($50 \mu\text{M}$) elicited a small inward current. Conditioned medium (CM) produced no significant response by itself, but markedly potentiated the effect of NMDA. Variability was observed in both the response to $50 \mu\text{M}$ NMDA alone (it was often smaller than shown here) and to CM alone (sometimes a response was observed, suggesting that the CM may contain some excitatory amino acids). In all cases the response to NMDA+CM was larger than the sum of the two separate responses.

Methods. Experiments were performed on primary cultures of cortical and diencephalic neurons from 15- to 16-day-old mouse embryos. The cells were kept in primary culture for up to 8 weeks as described²². Neurons generally adhered to a confluent lawn of glial cells on the culture dish bottom. Both the whole-cell recording mode and the outside-out mode of patch clamp recording²³ were used. Two different solutions were used in the patch pipette: (in mM) 140 CsCl, 5 EGTA-K, 0.5 CaCl_2 , 10 HEPES-K (CsCl solution); or 120 CsF, 10 CsCl, 10 EGTA-K, 10 HEPES-K (CsF solution); both pH 7.2. The NMDA response was the same with either internal solution. The external solution contained (in mM): 140 NaCl, 2.8 KCl, 1 CaCl_2 , 10 HEPES-Na (pH 7.2). Experiments were conducted at a temperature of 18 to 23 °C. The device used for rapid solution changes^{24,25} consisted of a double-barrelled glass tube (*a*). Control solution (barrel 1) and the solution to be tested (barrel 2) were fed by gravity from two independent reservoirs about 30 cm above the experimental chamber. The flow rate of about 0.1 ml min^{-1} resulted in a flow velocity of 5 cm s^{-1} through each $200\text{-}\mu\text{M}$ orifice. The boundary between the solutions flowing from the two barrels, which could be seen using solutions of different refractive index, was sharp for $>200 \mu\text{m}$ from the barrel exits. After the whole cell configuration²³ had been achieved the barrels were positioned within $100 \mu\text{m}$ of the cell so that the effluent of barrel 1 flowed over the neuron. The change of solution was accomplished by displacing under visual control the perfusion barrels, which were mounted on a micromanipulator. The use of this perfusion system assured that the cell under study was not exposed to the bath solution, into which potentiating factors may have been released. The exaggerated desensitization and 'off response'^{25,26} were thus avoided. The bath of the 35-mm culture dish was continuously perfused at low speed (0.5 to 2 ml min^{-1}) with external control solution to avoid accumulation of the compounds flowing continuously from the second barrel. Conditioned medium was prepared using the same type of cell cultures as used for physiological recordings. The medium in a culture dish was changed to the external solution by multiple washes. After an incubation of 2–8 h at room temperature, the conditioned medium was removed from the culture dish and stored frozen until used.

tested at $10 \mu\text{M}$, alanine and serine caused a small augmentation of the response to $10 \mu\text{M}$ NMDA, but were inactive when applied alone. No significant potentiation was observed with arginine, asparagine, cysteine, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, γ -amino butyric acid (GABA), hydroxyproline or cystine. The responses observed when perfusing $10 \mu\text{M}$ aspartate or glutamate (which activate NMDA receptors) with $10 \mu\text{M}$ NMDA could be explained as a summation of the action of these compounds on the NMDA receptor.

Preliminary high-performance liquid chromatography (HPLC) measurements of the glycine concentration in CM, gave

Transmitters mediating 'fast' synaptic processes in the vertebrate central nervous system are commonly placed in two separate categories that are believed to exhibit no interaction at the receptor level. The 'inhibitory transmitters' (such as glycine and GABA) are considered to act only on receptors mediating a chloride conductance increase, whereas 'excitatory transmitters' (such as L-glutamate) are considered to activate receptors mediating a cationic conductance increase¹. The best known excitatory receptor is that specifically activated by *N*-methyl-D-aspartate (NMDA)² which has recently been characterized at the single channel level^{3–5}. The response activated by NMDA agonists is unique in that it exhibits a voltage-dependent Mg block^{3,6}. We report here that this response exhibits another remarkable property: it is dramatically potentiated by glycine. This potentiation is not mediated by the inhibitory strychnine-sensitive glycine receptor^{7,8}, and is detected at a glycine concentration as low as 10 nM . The potentiation can be observed in outside-out patches as an increase in the frequency of opening of the channels activated by NMDA agonists. Thus, in addition to its role as an inhibitory transmitter, glycine may facilitate excitatory transmission in the brain through an allosteric⁹ activation of the NMDA receptor.

This investigation began with the observation that in cultured neurons the magnitude of the whole cell current produced by NMDA appeared to depend on the speed of perfusion of NMDA-containing solutions: slower movement of the perfusion solution resulted in a larger response (L. Nowak and P.A., unpublished data). One of many possible explanations for this observation is that a substance that augments the response to NMDA is tonically released from the cultured cells (neurons or glial), and accumulate when the perfusion is slow. To test this possibility, we compared the whole cell responses to fast perfusion of NMDA when dissolved in control Ringer or in conditioned medium (CM) (see Fig. 1, legend). In most experiments CM was observed to augment greatly the response to NMDA, but to produce little or no response by itself (Fig. 1*b*). The major active component of CM was observed to be resistant to heating to 90°C for 10 min, to pass through dialysis tubing of 3,500 relative molecular mass (M_r) cutoff, and to elute from a Sephadex G-10 column after the void volume. This last observation suggests that the major active component is of $M_r < 700$. In the search for low- M_r potentiating substances, the common amino acids were screened for their effect when applied in conjunction with NMDA. The effects of CM were reproduced by glycine.

The potentiation produced by glycine is illustrated in Fig. 2*a* for a concentration of $1 \mu\text{M}$, but it was detectable with 10 nM glycine. An augmentation of magnitude similar to that typically seen with CM was observed at a glycine concentration of $100\text{--}500 \text{ nM}$. Increasing the concentration from $1 \mu\text{M}$ to $10 \mu\text{M}$ increased the response by a factor of 1.1–1.2 (three experiments), indicating that the augmentation is near saturation at $1 \mu\text{M}$ glycine. The potentiation by NMDA is probably not due to a lateral shift of the NMDA dose-response curve, as there was little or no response to $1 \mu\text{M}$ NMDA even with $10 \mu\text{M}$ glycine, whereas $2 \mu\text{M}$ NMDA was occasionally sufficient to cause a response when applied alone. The mean inward current induced at -50 mV by $10 \mu\text{M}$ NMDA alone was 17 pA (range <3 to 40 , $n = 6$), whereas that induced by $10 \mu\text{M}$ NMDA + $1 \mu\text{M}$ glycine was 290 pA (range $50\text{--}500$, $n = 6$). When other amino acids were

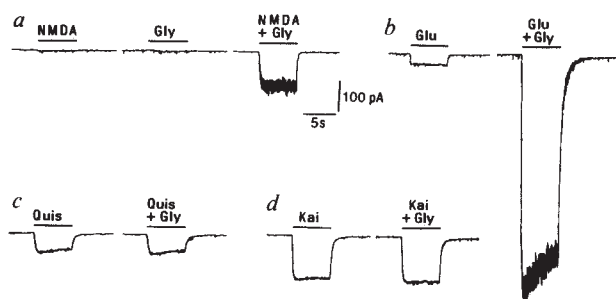


Fig. 2 Effects of 1 μ M glycine (Gly) on the currents induced by (a) 10 μ M NMDA, (b) 10 μ M L-glutamate (Glu), (c) 2 μ M quisqualate (Quis), and (d) 10 μ M kainate (Kai). Whole cell recording. Internal solution, CsCl. Holding potential, -50 mV. Glycine potentiates the currents produced by NMDA or glutamate, and does not potentiate the currents produced by quisqualate or kainate. In this experiment, neither 1 μ M glycine alone nor 10 μ M NMDA alone induced a significant current, whereas a large response was produced when they were applied together. In some cells a response to 10 μ M NMDA alone was observed (Fig. 3a, for example) whereas the response to 1 μ M glycine alone was consistently very small (Fig. 3b) or negligible. The quisqualate- and kainate-induced currents show little noise, as expected from the fact that the single channel conductances of the corresponding 'non-NMDA' channels are smaller than that of the NMDA channels^{27,5}. In the absence of glycine, the noise of the glutamate-induced current is of similar magnitude to that of the quisqualate- and kainate-induced currents, suggesting that most of the current flows through non-NMDA channels. In the presence of glycine the noise variance increases dramatically, indicating the recruitment of NMDA channels.

a value of $0.32 \pm 0.16 \mu$ M (mean $\pm$ s.d., 5 different CMs) (Dr J.-P. Pin, personal communication). This concentration of glycine appears sufficient to account for the potentiation observed with the corresponding conditioned media.

The effect of glycine is specific to the NMDA receptor. Glycine augmented the response to glutamate as well as that to NMDA (Fig. 2b), but the inward currents induced by quisqualate or kainate, two putative glutamate agonists that do not act on the NMDA receptor², were essentially unaffected by glycine (Fig. 2c, d). The same pattern of specificity was observed with CM. The current produced by application of NMDA with glycine exhibits the same voltage-dependent Mg block previously described^{3,6} for the NMDA-induced current (data not shown).

The action of glycine on the NMDA response does not involve the strychnine-sensitive receptor that mediates the classical inhibitory glycine response^{7,8,10,11}. The potentiation produced by 1 μ M glycine was not blocked by strychnine at 1 μ M or 10 μ M (Fig. 3a). In the same cell a chloride (Cl) conductance increase was not observed in response to perfusion of 1 μ M glycine (a concentration at which the potentiation of the NMDA response is nearly maximal), but was observed during application of 10 μ M glycine (Fig. 3b). This Cl conductance increase (characterized at a potential of -20 mV by an outward current under the experimental conditions of Fig. 3) was blocked by 1 μ M strychnine. Glycine induced a Cl conductance increase in only a few cells, whereas the augmentation of the NMDA response by glycine was observed in all cells studied.

The potentiating effect of glycine on the NMDA response was also observed in outside-out patches. The potentiation was due predominantly to an increase in the frequency of channel opening (Fig. 4). In some patches, the increase in opening frequency could not be calculated accurately because there were too few openings in 10 μ M NMDA alone. In those outside-out patches for which it was calculable, the mean increase in frequency was 9.1 (range 1.9–25, $n=5$). The amplitude of the single channel current was the same in NMDA alone as in NMDA + glycine (Fig. 4b). A small augmentation of the mean

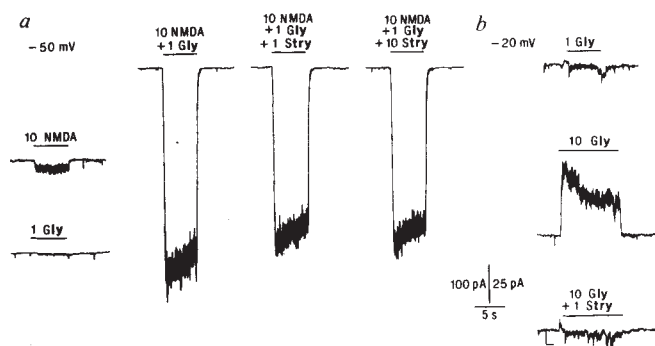


Fig. 3 Strychnine (Stry) does not block the glycine-induced potentiation of the NMDA response but does block the glycine-induced anionic conductance. Whole cell recording. Internal solution, CsF. *a*, At a holding potential of -50 mV the response to 10 μ M NMDA is markedly potentiated by 1 μ M glycine. The potentiation persists after addition of 1 μ M or 10 μ M strychnine. The small reduction of the response in strychnine was probably not due to the addition of this compound, because after removing strychnine the response to NMDA + glycine was somewhat smaller (data not shown) than in strychnine. *b*, At a holding potential of -20 mV glycine at 1 μ M elicits a slight increase in noise which at high gain appears to be constituted of transient inward currents resembling synaptic currents. These currents are similar to events occurring at lower frequency before glycine application. They may represent synaptic currents mediated by NMDA receptors and potentiated by glycine. At 10 μ M, glycine elicited an outward current, which inverted at near -40 mV (data not shown) and was completely blocked by 1 μ M strychnine. A small inward current similar to that seen in 1 μ M glycine remained. Given the composition of the extracellular and intracellular solutions, the outward current is likely to be carried largely by Cl (the only other ion with a negative reversal potential is Cs) through a channel with some permeability to F (the main intracellular anion). Note that the reversal potential measured in these experiments was more negative than that found in similar conditions, but in outside-out patches, by Hamill *et al.*¹¹. This may indicate an incomplete exchange of the internal ions. Note difference in current gain between *a* and *b*.

open time by glycine was measured in 4 of the 5 experiments analysed (see Fig. 4c). The mean open time in NMDA alone varied from 5.8 to 8.2 ms, whereas in NMDA + glycine the range was 6.3–12.3 ms.

The involvement of glycine in the NMDA response makes difficult the interpretation of results from experiments in which NMDA is applied to a neuron without complete control of the external milieu, because of the possible release of glycine by surrounding cells. Some of the discrepancies in the literature suggesting that the NMDA response is more easily observed in brain slice preparations than in isolated neurons¹² may be due to an inability to remove glycine from around neurons in slices. Similarly, some of the variability observed in the NMDA responses of cultured neurons may be related to the type of perfusion used; in primary cultures as used here, glycine appears to be released at a rate sufficient to augment the response to NMDA unless a fast perfusion method is used. In examining this variability it may also be important to note the type of culture medium used²⁸. The medium most commonly used for the culture of central neurons, Eagle's minimal essential medium (MEM), differs from other commonly used culture media in that it lacks glycine. Long term exposure to glycine may influence the response of a neuron to NMDA.

The functional importance of the glycine potentiation of the NMDA response will depend on whether the potentiation exists in mature neurons *in vivo*, and whether glycine and an endogenous NMDA agonist such as glutamate are present simultaneously at NMDA receptors. Because both glycine and glutamate are present in the cerebrospinal fluid at $>1 \mu$ M (ref. 13),

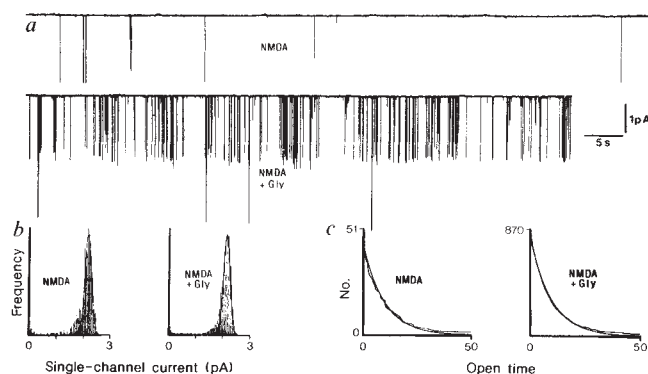


Fig. 4 Glycine potentiation of NMDA response in an outside-out patch. Internal solution, CsF. Holding potential, -50 mV. *a*, Record of the single channel currents produced by $10 \mu\text{M}$ NMDA (upper record) and $10 \mu\text{M}$ NMDA + $1 \mu\text{M}$ glycine (lower record). The mean frequency of channel openings was 0.16 s^{-1} in the absence of glycine, and 4.0 s^{-1} in the presence of glycine. No NMDA channel openings were observed during application of $1 \mu\text{M}$ glycine alone (not illustrated). Most of the smaller deflections correspond to short openings that were not faithfully reproduced due to low-pass filtering used in the figure (effective filter frequency, 200 Hz). *b*, Amplitude histograms of the single channel currents in NMDA (left) and NMDA + glycine (right), obtained by placing a window around the openings and measuring the amplitude of the current during the openings. The points near the zero of the abscissa correspond to closures of the channel during the duration defined by the window. The gaussian curves fitted to the histograms have a mode of 2.16 pA for NMDA and 2.13 pA for NMDA + glycine. The single channel conductance of 43 pS is within the range previously observed for the main conductance state of the NMDA channel³. *c*, Cumulative open time histograms of the channels in NMDA (left) and in NMDA + glycine (right). Both histograms were well fitted with an exponential having a time constant of 10 ms . However, the arithmetic mean open time was slightly different between NMDA (8.2 ms) and NMDA + glycine (10.0 ms). The numbers given in the text correspond to the arithmetic mean duration. The figures at the top of each ordinate indicate the total number of openings in the sample.

they may be present in the extracellular space at concentrations sufficient to activate an NMDA response. Thus, if a neuron possesses glycine-sensitive NMDA receptors, these may be constantly activated. In physiological concentrations of extracellular Mg (1 mM) this will not result in a marked cell depolarization, but will endow neurons expressing numerous NMDA receptors with a strong voltage dependence, making them prone to the oscillatory behaviour associated with NMDA activation in physiological conditions^{14,15} and in some pathological states such as epilepsy¹⁶. Because such a high percentage of central neurons respond to NMDA, variation in the concentrations of glycine and excitatory amino acids could influence the excitability of neurons involved in a wide range of functions.

The glycine-glutamate synergism may also be of importance in phasic phenomena: a steady-state background of glycine may potentiate the effect of a pulse of glutamate released at a glutamatergic synapse; a steady-state glutamate background around an inhibitory glycinergic synapse may lead to a delayed excitation of the same cell or of nearby neurons if NMDA receptors are present; the release of glycine and glutamate from separate terminals in close proximity could result in a level of NMDA channel activation very sensitive to the timing of input arrival. A possible candidate for such glutamate-glycine interactions is the lateral dendrite of the Mauthner cell, which is known to be excited by glutamate and inhibited by glycine. Large vesicle boutons and small vesicle boutons, presumed to correspond to excitatory and inhibitory terminals, are closely intertwined on this dendrite¹⁷.

The molecular mechanism of the potentiation by glycine remains to be established. Because the effect can be observed in outside-out patches and because the inward current produced by NMDA + glycine in whole cells follows application of agonists with little delay, an intracellular relay cannot be involved. One possibility is that of an allosteric modulation similar to that assumed to occur between the benzodiazepine receptor and the GABA receptor-channel complex¹⁸⁻²¹. It is not clear at present whether the glycine binding site and the NMDA binding site are on distinct proteins, but if they are the two proteins must be able to interact directly. The NMDA receptor appears to be an original example of a receptor-channel system with rapid dual regulation: a negative control exerted by Mg , and a positive control mediated by glycine.

We thank M. Dennis, L. Nowak, A. Marty, R. Horn, I. Llano, A. Trautmann and D. Levy for their help. Support was provided by CNRS (UA 295), INSERM (CRE 856001), Université Pierre et Marie Curie and Fondation pour la Recherche Médicale. J.W.J. was supported by the NSF-CNRS exchange program.

Received 10 November; accepted 11 December 1986.

- Iversen, L. L. *Proc. R. Soc. B* **221**, 245-260 (1984).
- Watkins, J. C. & Evans, R. H. A. *Rev. Pharmac.* **21**, 165-205 (1981).
- Nowak, L., Bregestovski, P., Ascher, P., Herbet, A. & Prochiantz, A. *Nature* **307**, 462-465 (1984).
- Cull-Candy, S. G. & Usowicz, M. M. *Nature* **325**, 525-528 (1987).
- Jahr, C. E. & Stevens, C. F. *Nature* **325**, 522-525 (1987).
- Mayer, M. L., Westbrook, G. L. & Guthrie, P. B. *Nature* **309**, 261-263 (1984).
- Curtis, D. R., Hösl, L. & Johnston, G. A. R. *Exp. Brain Res.* **6**, 1-18 (1968).
- Graham, D., Pfeiffer, F., Simler, R. & Betz, H. *Biochemistry* **24**, 990-994 (1985).
- Monod, J., Wyman, J. & Changeux, J. P. *J. molec. Biol.* **12**, 88-118 (1965).
- Werman, R., Davidoff, R. A. & Aprison, M. H. *Nature* **214**, 681-683 (1967).
- Hamill, O. P., Bormann, J. & Sakmann, B. *Nature* **305**, 805-807 (1983).
- Kiskin, N. I., Krishtal, O. A. & Tsytrendenko, A. Ya. *Neurosci. Lett.* **63**, 225-230 (1986).
- Ferraro, T. N. & Hare, T. A. *Brain Res.* **338**, 53-60 (1985).
- Sigwardt, K. A., Grillner, S., Wallen, P. & Van Dongen, P. A. M. *Brain Res.* **336**, 390-395 (1985).
- Herrling, P. L., Morris, R. & Salt, T. E. J. *Physiol., Lond.* **339**, 207-222 (1983).
- Fagg, G. E. *Trends Neurosci.* **8**, 207-210 (1985).
- Nakajima, Y. *J. comp. Neurol.* **156**, 375-402 (1974).
- Choi, D. W., Farb, D. H. & Fischbach, G. D. *Nature* **269**, 342-344 (1977).
- Study, R. E. & Barker, J. L. *Proc. natn. Acad. Sci. U.S.A.* **78**, 7180-7184 (1981).
- Bormann, J. & Sakmann, B. *IUPHAR 9th int. Congr. Pharmac. Abstr.* S13-14 (Macmillan, London, 1984).
- Haefely, W. E., Kyburz, E., Gerecke, M. & Möhler, H. *Adv. Drug. Res.* **14**, 165-322 (1985).
- Ransom, B. R., Neale, E., Henkart, M., Bullock, P. N. & Nelson, P. G. *J. Neurophysiol.* **40**, 1132-1150 (1977).
- Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Pflügers Arch. ges. Physiol.* **391**, 85-100 (1981).
- Yellen, G. *Nature* **296**, 357-359 (1982).
- Johnson, J. & Ascher, P. *Soc. Neurosci. Abstr.* **12**, 58 (1986).
- Vyklicky, L. *et al. Brain Res.* **363**, 148-151 (1986).
- Ascher, P., Nowak, L. & Kehoe, J. S. in *Ion channels in Neural Membranes* (eds Ritchie, J. M., Keynes, R. D. & Bolis, L.) 283-295 (Liss, New York, 1986).
- Nelson, P. G., Ransom, B. R., Henkart, M. & Bullock, P. N. *J. Neurophysiol.* **40**, 1178-1187 (1977).

A new type of glutamate receptor linked to inositol phospholipid metabolism

Hiroyuki Sugiyama, Isao Ito
& Chikara Hirono

Department of Cellular Physiology, National Institute for Physiological Sciences, Myodaiji, Okazaki 444, Japan

Receptors for excitatory amino acids in the mammalian central nervous system are classified into three major subtypes^{1,2}, ones which prefer *N*-methyl-D-aspartate (NMDA), quisqualate (QA), or kainate (KA) as type agonists respectively. These receptors are considered to mediate fast postsynaptic potentials by activating ion channels directly³⁻⁵ (ionotropic type⁶). Recently it was reported that exposure of mammalian brain cells to glutamate (Glu) or its analogues causes enhanced hydrolysis of inositol phospholipids^{7,8}, but it is not clear whether the enhanced hydrolysis is the cause or effect of physiological responses. Membrane depolarization or Ca^{2+} influx, which can result from Glu receptor activation^{9,10}, can

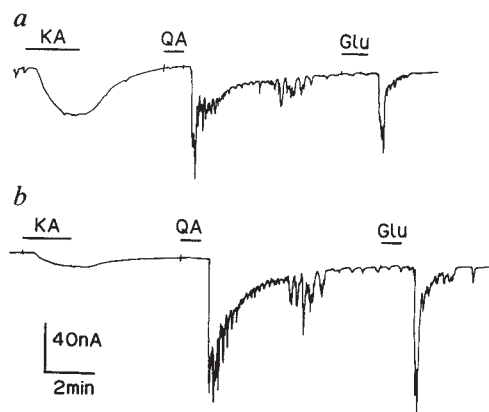


Fig. 1 Typical current responses of *Xenopus* oocytes injected with rat-brain mRNA to bath-applied KA, QA and Glu before (a) and after (b) treatment with JSTX. After recording the responses in a, the oocyte was treated with JSTX (0.1 unit ml^{-1} or 0.01 u (ref. 16)) for 6 min and washed briefly. Then the drugs were applied in the absence of the toxin. KA responses were strongly inhibited, whereas QA/Glu responses were not suppressed (or were even slightly potentiated). From 12 experiments, maximum current amplitudes of KA response, relative to those before JSTX treatment, were found to be $19 \pm 3\%$ (mean $\pm$ s.e.m.). Downward deflections represent inward currents and horizontal bars, the time of drug application. Concentrations used were: KA, $100 \mu\text{M}$; QA and Glu, $10 \mu\text{M}$.

Methods. The mRNA was extracted from whole brain of adult rats, purified by oligo(dT) affinity chromatography, and size-fractionated by sucrose gradient centrifugation^{17,22}. Fractions corresponding to 20–40 S RNA were used. Oocytes were treated with collagenase, injected with 100 ng of poly(A)⁺ mRNA, and incubated for 2–3 days as described^{17,21,22}. Under voltage clamp conditions (-60 mV), current response of the oocytes were recorded in normal Ringer (composition in mM: NaCl, 90; KCl, 2; CaCl_2 , 2; Tris-HCl, 10; pH 7.6) at $22\text{--}25^\circ\text{C}$.

induce enhanced hydrolysis of inositol phospholipids¹¹. We have characterized the functional properties of two types of excitatory amino-acid responses, those activated by QA (or Glu) and those activated by KA, induced in *Xenopus* oocytes injected with rat-brain messenger RNA¹². We report evidence for a new type of Glu receptor, which prefers QA as agonist, and which directly activates inositol phospholipid metabolism through interaction with GTP-binding regulatory proteins (G_i or G_o ^{13,14}), leading to the formation of inositol 1,4,5-trisphosphate (InsP_3) and mobilization of intracellular Ca^{2+} . This QA/Glu reaction is inhibited by islet-activating protein (IAP, pertussis toxin¹⁵), but was not blocked by Joro spider toxin (JSTX)¹⁶, a specific blocker of traditional ionotropic QA/Glu receptors.

Membrane current responses were observed to bath-applied QA or KA in *Xenopus* oocytes injected with rat brain mRNA (Fig. 1a; ref. 12). NMDA ($\leq 1 \text{ mM}$) caused no response, indicating that NMDA-preferring receptors were not strongly expressed in the oocytes. Glu evoked essentially the same responses as QA, although its potency was ~ 10 times lower (Fig. 2). Current responses to QA (or Glu) showed characteristics very different from those of QA responses generally observed in crustacean neuromuscular junctions or mammalian brains. These QA/Glu responses were fluctuating and had delayed onset, suggesting the involvement of intracellular metabolic processes, and, when mRNA-injected oocytes were treated with IAP, were significantly reduced (Fig. 3a), indicating that QA/Glu responses are mediated by G_i or G_o proteins.

The involvement of InsP_3 in the reaction mechanism of QA/Glu responses was suggested by experiments in which InsP_3 was injected into the oocytes. Intracellularly injected InsP_3 evoked current responses similar to QA/Glu responses (Fig. 4a). When stimulated by a large dose of InsP_3 , however, the oocyte became 'desensitized', and a second injection caused no

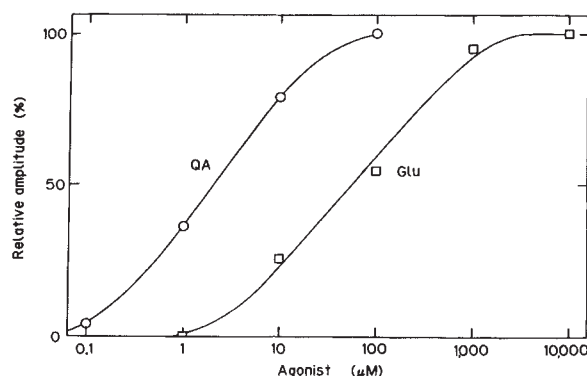


Fig. 2 Dose-response curves of QA or Glu-induced inward currents in mRNA injected oocytes. Oocytes were exposed to increasing concentrations of each amino acid. Results are means of two different experiments, expressed as percentage of the response induced by 10 mM Glu or $100 \mu\text{M}$ QA. The holding potential was -60 mV . At high concentration ($\geq 1 \text{ mM}$), Glu induced inward currents of both QA-type (fluctuating) and KA-type (smooth). The contribution was estimated to be less than 10% . NMDA (1 mM), KA (0.5 mM), DL-2-amino-4-phosphonobutyric acid (APB) (1 mM) and DL-2-amino-5-phosphonovaleic acid (APV) (0.5 mM) neither activated nor inhibited QA-type responses. Glutamate diethyl ester (GDEE) ($< 1 \text{ mM}$) had no inhibitory effect, but behaved as a weak agonist at higher concentrations ($\geq 1 \text{ mM}$).

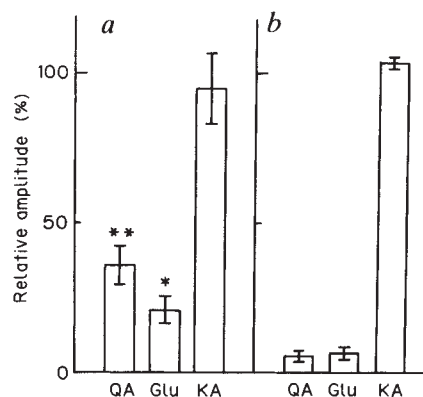


Fig. 3 Inhibition by IAP (a) and intracellular EGTA (b) of oocyte responses to QA and Glu. a, Oocytes were injected with 100 ng mRNA, incubated for 2 days, then further incubated for 20 h in the presence or absence of IAP ($2 \mu\text{g ml}^{-1}$) at 20°C . Maximum amplitudes of current responses are shown relative to the values of control (no IAP) oocytes. Columns, mean; bars, $\pm$ s.e.m. range; $n = 13$. *, $P < 0.001$; **, $P < 0.01$ by t -test. b, Each oocyte, injected with 100 ng mRNA and incubated for 4 days, was exposed to QA, Glu or KA, and the response was recorded. After extensive washing, EGTA was injected¹⁷ to a final concentration of $170 \mu\text{M}$, and 3 min later, the response to the same stimulant was recorded. The maximum current amplitude relative to that before EGTA is shown as mean $\pm$ s.e.m. for $n = 4$. Concentrations used were: QA and Glu, $10 \mu\text{M}$; KA, $100 \mu\text{M}$ (a and b).

response¹⁷. Under such conditions, the current responses to QA/Glu were found to be greatly reduced (Fig. 4a), whereas responses to KA were not affected¹⁷, suggesting that intracellular InsP_3 desensitized specifically QA/Glu responses. InsP_3 is considered to be an intracellular messenger for intracellular Ca^{2+} mobilization¹⁸, and its involvement in our QA/Glu responses was supported by the observation that intracellular injection of EGTA inhibited QA/Glu responses as well as responses to InsP_3 injection¹⁷, without affecting KA responses (Fig. 3b). These characteristics of our QA/Glu responses are almost identical to those of muscarinic acetylcholine (ACh) responses and

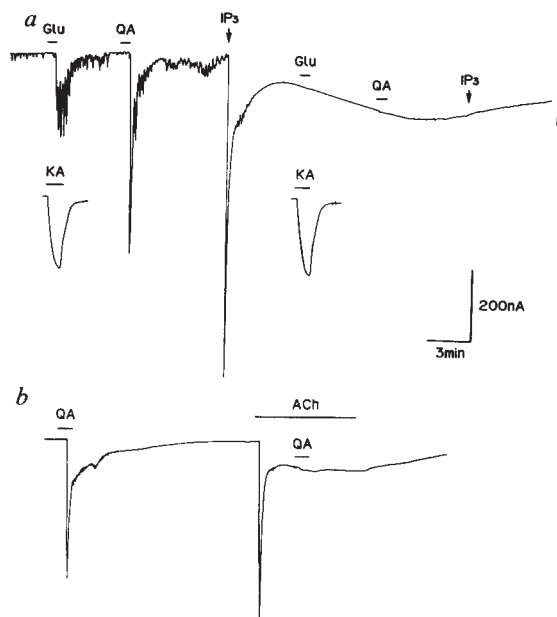


Fig. 4 *a*, Effects of intracellular injection of InsP₃ and *b*, 'cross-desensitization' between QA and ACh responses. In *a*, oocytes injected with 100 ng mRNA and incubated for 2 days were exposed to 10 μ M QA, 10 μ M Glu or 300 μ M KA (bars), or injected with 30 pmol InsP₃ (arrows). The first injection rendered the oocyte refractory to Glu, QA or InsP₃, but KA responses were unaffected. In *b*, an oocyte injected with 100 ng mRNA and incubated for 3 days was first stimulated with 10 μ M QA. After 12 min washing, the oocyte was exposed to 200 μ M ACh. Under such conditions, a second application of 10 μ M QA produced no appreciable responses. Desensitization of ACh responses by QA was also observed (data not shown).

neurotensin responses which are also induced¹⁷ in oocytes by rat-brain mRNA, suggesting that all these responses are mediated by a common intracellular mechanism. This is supported by the observations that; 1, reversal potentials of QA/Glu responses are identical to those of ACh or neurotensin responses and determined by Cl⁻ ions (data now shown), so that activation of all these receptors results in increases in Cl⁻ conductance, and 2, QA/Glu responses and ACh responses show 'cross-desensitization': when an oocyte was desensitized by a large dose of ACh, the responsiveness of the cell to QA (or Glu) was greatly suppressed, and vice versa (Fig. 4b). We conclude that QA/Glu receptors, like muscarinic ACh receptors^{17,19}, activate G_i or G_o proteins, leading to formation of InsP₃ and intracellular Ca²⁺ mobilization. This is the first direct evidence for the presence of a metabotropic type of glutamatergic receptor working through a second messenger system. Such QA/Glu responses were never observed with native oocytes (no mRNA injected), although similar responses to InsP₃ injection were observed both in mRNA-injected and native oocytes¹⁷, so the receptors were most probably induced by rat-brain mRNA. This strongly suggests the existence of such metabotropic Glu receptors in the brain, although the final effector of this receptor in brain may not necessarily be the Cl⁻ channels observed in our oocyte system.

Although the metabotropic Glu receptor can be activated by QA but not KA or NMDA, this receptor is pharmacologically different from traditional QA-preferring receptors in important aspects. Glutamate diethyl ester, a typical specific antagonist to QA-preferring receptors^{1,2}, shows no inhibitory effects on this receptor (≤ 1 mM). JSTX, a spider toxin known to block ionotropic QA/Glu transmission in crustacean muscles¹⁶ and mammalian brains²⁰, causes no inhibition on metabotropic QA/Glu responses (Fig. 1).

Instead, JSTX inhibited KA responses (Fig. 1). KA responses were totally insensitive to the intracellular manipulations men-

tioned above (Figs 3 and 4). They show characteristics very similar to those of nicotinic ACh responses, namely short latency, smooth pattern, and reversal potential close to 0 mV (C.H., I.I. & H.S., in preparation), and can be considered to be ionotropic. Glu also caused KA-type responses but at rather high concentrations (≥ 1 mM). One of possible explanations is that, as has been observed in rat brain neurons⁵, Glu, but not KA, may cause quick desensitization of the receptors so that it may be difficult to detect responses to Glu applied slowly by perfusion. Whatever may be the case, JSTX appears to block ionotropic non-NMDA glutamatergic responses, but has no inhibitory effects on metabotropic QA/Glu responses. Thus JSTX and IAP may provide new criteria for classification of excitatory amino-acid receptors based upon functional properties rather than pharmacological properties.

We are grateful to Professor M. Ui for IAP and to Dr N. Kawai for JSTX. After completion of this work, we were informed that Dr R. Miledi and collaborators had observed a blockade of KA responses in a similar oocyte system using JSTX of less purified grade (N. Kawai, R. Miledi & K. Sumikawa, personal communication), and we thank Dr N. Kawai for his communication and encouragement.

Received 24 October; accepted 12 December 1986.

1. Watkins, J. C. & Evans, R. H. *A. Rev. Pharmac. Toxicol.* **21**, 165-204 (1981).
2. Fagg, G. E. *Trends Neurosci.* **8**, 207-210 (1985).
3. Nowak, L., Bregestovski, P., Ascher, P., Herbet, A. & Prochiantz, A. *Nature* **307**, 462-465 (1984).
4. Cull-Candy, S. G. & Ogden, D. C. *Proc. R. Soc. B224*, 367-373 (1985).
5. Kiskin, N. I., Krishtal, O. A. & Tsyndrenko, A. Ya. *Neurosci. Lett.* **63**, 225-230 (1986).
6. Eccles, J. C. & McGeer, P. L. *Trends Neurosci.* **2**, 39-40 (1979).
7. Sladeczek, F., Pin, J. P., Recasens, M., Bockaert, J. & Weiss, S. *Nature* **317**, 717-719 (1985).
8. Nicoletti, F. *et al. J. Neurochem.* **46**, 40-46 (1986).
9. Retz, K. C. & Coyle, J. T. *Neuropharmacology* **23**, 89-94 (1984).
10. MacDermott, A. B., Mayer, M. L., Westbrook, G. L., Smith, S. J. & Barker, J. L. *Nature* **321**, 519-522 (1986).
11. Kendall, D. A. & Nahorski, S. R. *J. Neurochem.* **42**, 1388-1394 (1984).
12. Gundersen, C. B., Miledi, R. & Parker, I. *Proc. R. Soc. B221*, 127-143 (1984).
13. Sternweis, P. C. & Robishaw, J. D. *J. biol. Chem.* **259**, 13806-13813 (1984).
14. Neer, E. J., Lok, J. M. & Wolf, L. G. *J. biol. Chem.* **259**, 14222-14229 (1984).
15. Ui, M. *Trends pharmac. Sci.* **5**, 277-279 (1984).
16. Abe, T., Kawai, N. & Miwa, A. *J. Physiol., Lond.* **339**, 243-252 (1983).
17. Hirono, C., Ito, I. & Sugiyama, H. *J. Physiol., Lond.* **382**, 523-535 (1987).
18. Berridge, M. J. & Irvine, R. F. *Nature* **312**, 315-321 (1984).
19. Oron, Y., Dascal, N., Nadler, E. & Lupu, M. *Nature* **313**, 141-143 (1985).
20. Saito, M., Kawai, N., Miwa, A., Pan-Hou, H. & Yoshioka, M. *Brain Res.* **346**, 397-399 (1985).
21. Sugiyama, H., Hisanaga, Y. & Hirono, C. *Brain Res.* **338**, 346-350 (1985).
22. Hirono, C. *et al. Brain Res.* **359**, 57-64 (1985).

Platelets mediate the action of diethylcarbamazine on microfilariae

Jean-Yves Cesbron*, André Capron*, Boris B. Vargaftig†, Michel Lagarde‡, Joël Pincemail§, Pierre Braquet||, Henri Taelman¶ & Michel Joseph*

* Centre d'Immunologie et de Biologie Parasitaire, Unité Mixte INSERM 167-CNRS 624, Institut Pasteur, 1 rue du Pr A. Calmette, 59019 Lille Cédex, France

† Unité des Venins, Unité Associée Institut Pasteur-INSERM 285, Institut Pasteur, 25 rue du Dr Roux, 75015 Paris, France

‡ Laboratoire d'Hémobiologie, Unité INSERM 63, Institut Pasteur, Faculté Alexis Carrel, 69372 Lyon Cédex 08, France

§ Unité de Biochimie de l'Oxygène, Université de Liège, Bâtiment de Chimie B 6, Sart Tilman, 4 000 Liège, Belgium

|| Institut Henri Beaufour, 17 avenue Descartes, 92350 Le Plessis, France

¶ Institute for Tropical Medicine, 155 Nationalestraat, 2000 Antwerpen, Belgium

More than 400 million people in the world are infected by filarial parasites leading to a wide range of pathologies¹. Although introduced in 1947², the mainstay of the therapy and control of the filariases is diethylcarbamazine (*N,N*-diethyl-4-methyl-1-piperazine carboxamide; DEC), the mode of action of which still remains unknown despite widespread use and intensive laboratory

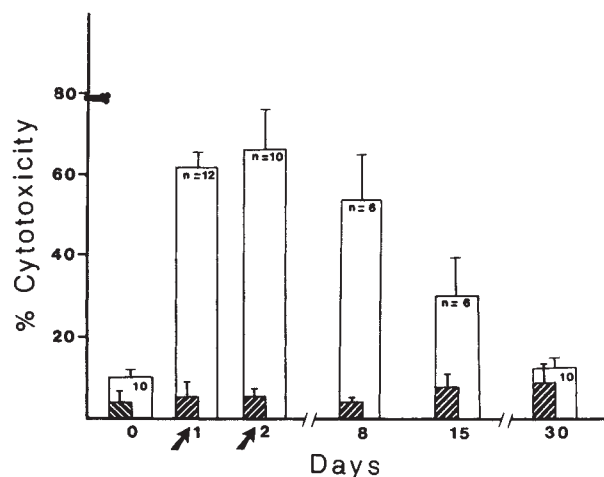


Fig. 1 Cytotoxic activity against *L. loa* microfilariae of platelets isolated from healthy donors after oral doses of DEC. The larvae, collected from the blood of highly infected patients by discontinuous flow centrifugation⁸, after coagulation of the collected samples, were isolated by membrane filtration (Sartorius; 8.0 μ m diameter) of the sera obtained. DEC (Specia) was administered to 3 volunteers at a dosage of 25 mg and 75 mg on days 1 and 2 respectively (arrow). The cytotoxic activity was tested from the day before the first dose until one month later. The blood (6 vol.) was collected on ACD-C²⁵ anticoagulant (1 vol.) 3 h after oral administration. After purification^{6,7}, 7.5×10^7 platelets in 100 μ l of EMEM were incubated in flat-bottomed microplates (Nunc) with 20 μ l of serum from healthy donors and ~ 100 microfilariae in 80 μ l EMEM. The cytotoxic activity was tested after 18 h at 37°C in 5% CO₂ in air. The dying larvae became granular, stiff and immobile, and after 18 h were undetectable by optical microscopy; only the living, mobile and refringent microfilariae were counted. The number of larvae in each well was determined before assay, and each experiment was performed in duplicate. The results are expressed as mean $\pm$ s.e.m. of per cent dead larvae. Shaded bars, cytotoxicity of the sera on larvae without platelets (control). The significance was determined by a two-tailed analysis of variance, showing a significant difference for the period pre- and post-DEC administration ($F = 25.7$; $P < 0.05$) with no difference between individuals ($F = 16.6$; $P > 0.05$). The participation of contaminant cells (150 in each assay after gentian violet staining) was ruled out, as leukocytes—made up of 50% adherent mononuclear cells and 50% granulocytes (>95% neutrophils)—purified after removal of platelet-rich plasma, showed no significant cytotoxic activity in the presence of DEC. Moreover, the addition, into the assay, of 5×10^4 or 5×10^5 total leukocytes to suspensions of platelets (in suboptimal concentrations) did not change the percentage of killing.

investigations¹⁻³. The marked contrast between an extremely rapid action *in vivo*³⁻⁴ and the absence of any significant activity on microfilariae *in vitro* is unique among chemotherapeutic agents. DEC has been thought to modify the surface layer of the microfilariae and expose them to immunological cell-mediated lysis⁵. This report provides the first evidence that the effect of DEC is mediated by blood platelets with the additional triggering of a filarial excretory antigen (FEA). The killing mechanism is antibody-independent and involves the participation of free radicals.

Initially, the effect of DEC on platelets was evaluated *in vivo* after oral administration. Platelets purified⁶⁻⁷ from the blood of healthy volunteers three hours after the ingestion of 25 mg DEC (the time corresponding to the peak blood level of the drug³) and incubated with *Loa loa* microfilariae, showed strong cytotoxic activity (Fig. 1). Higher doses or repeated administration of DEC did not significantly increase the parasite killing which was maintained for more than a week and slowly returned to the control rate after 15 days (Fig. 1). The participation of leukocytes in the process was ruled out (Fig. 1, legend).

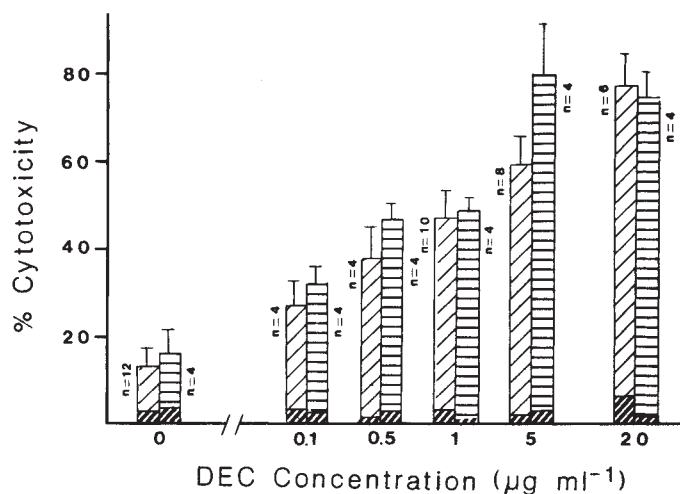


Fig. 2 Cytotoxic activity of platelets isolated from normal healthy donors and treated *in vitro* with increasing doses of DEC, against *B. malayi* microfilariae produced *in vitro*. Light diagonal shading, cytotoxicity in assays containing DEC + 10% normal fresh serum in EMEM; horizontal shading, assays containing DEC in EMEM alone; heavy diagonal shading each medium without platelets. Microfilariae, produced *in vitro* by gravid females from the peritoneal cavity of infected jirds (*Meriones unguiculatus*), were kept in culture in a serum-free medium (RPMI 1640, GIBCO-CULT¹⁰). Microfilariae were collected after 48 h of culture, washed in EMEM, concentrated by gentle centrifugation and adjusted to the appropriate concentration before the cytotoxicity assay. Pure DEC (batch 24 40537 Specia) was dissolved in EMEM, sterilized by 0.22 μ m membrane filtration (Millipore) and diluted to the appropriate final concentration in 10% normal fresh sera in EMEM or in EMEM alone. The pH was maintained at 7.2 and the cytotoxicity assay was as described in Fig. 1.

L. loa microfilariae were isolated from infected patients⁸, so specific antibodies could be adsorbed onto the surface of the larvae⁵ thus accounting for the cytotoxicity⁹. To examine this possibility, *Brugia malayi* microfilariae produced *in vitro* from gravid female cultures in a serum-free medium¹⁰ were incubated in Eagle's minimum essential medium (EMEM) with platelets from untreated volunteers. The addition of DEC from 0.1 μ g ml⁻¹ up to 20 μ g ml⁻¹ led to a dose-dependent cytotoxic effect (Fig. 2), without platelet lysis, as assessed by optical microscopy and by the absence of lactate dehydrogenase in platelet supernatants.

Therefore, our data do not support a role for complement¹¹ or antibodies⁵⁻¹². At a constant concentration of DEC, the cytotoxicity was directly related to the number of platelets present in the test. It is noteworthy that 1, platelets treated *in vitro* by DEC down to 0.5 μ g ml⁻¹, reported as the minimum effective serum concentration³, were cytotoxic; and 2, we failed to observe a direct lethal effect of DEC on *L. loa* and *B. malayi* microfilariae, in accordance with the current opinion that the action of DEC is not related to a chemical toxicity of the drug or of any of its metabolites^{3,4}. There is, however, indirect experimental evidence that platelets are needed for the microfilaricidal effect of DEC, such as its failure to be active in platelet-free compartments (for instance, the pleural cavity) even at high concentrations³⁻⁴. The initial observation (Fig. 1) that, 15 days after oral administration of DEC, platelets alone were active against microfilariae whereas free DEC is undetectable after 48 hours⁴ suggested that the targets of DEC were platelets and, probably, their bone marrow precursors as the effect of DEC outlasted the average platelet life-time of 9-10 days.

The demonstration that the platelets treated with DEC killed microfilariae raised the question of their target specificity. Indeed, such platelets were inactive against *Schistosoma mansoni* schistosomula, unless living microfilariae were introduced into

Table 1 Effect of FEA on the cytotoxic action of DEC-treated platelets against *S. mansoni* schistosomula

Platelets incubated with	% Cytotoxicity against schistosomula	
DEC	9.0±2.0	n = 6
DEC+FEA	73.0±4.0	
FEA	13.0±2.0	

FEA was isolated by immunoadsorption from the culture medium of *B. malayi* adult worms using a monoclonal antibody previously described as recognizing the surface of *B. malayi* microfilariae²⁶. The cytotoxicity assay was performed with 20 µg ml⁻¹ DEC and 5 µg ml⁻¹ FEA against *S. mansoni* schistosomula²¹. The absence of lysis of platelets treated simultaneously with DEC and FEA was appreciated by the level of the cytoplasmic marker lactate dehydrogenase in the reaction supernatant (LDH Biochemica test, Boehringer). Neither DEC or FEA, alone or together, induced any aggregation of the platelets.

Table 2 Effect of inhibitors of arachidonate pathways on the platelet/DEC cytotoxicity against *B. malayi* microfilariae

Platelets incubated with	% Cytotoxicity against <i>B. malayi</i> microfilariae	
DEC	82±3	n = 22
DEC+0.3×10 ⁻³ M aspirin	95±2	
0+0.3×10 ⁻³ M aspirin	14±4	n = 4
DEC+10 ⁻⁶ M NDGA	15±1	
0+10 ⁻⁶ M NDGA	12±1	n = 2
DEC+10 ⁻⁶ M Esculetin	15±3	
0+10 ⁻⁶ M Esculetin	10±2	n = 8
DEC+5×10 ⁻⁶ M 5,8,11,14-(21=4)	19±4	
0+5×10 ⁻⁶ M 5,8,11,14-(21=4)	15±12	n = 4

Acetyl-salicylic acid (aspirin) (Theraplix Laboratories, Paris, France) and 6,7-dihydroxy-coumarin (Esculetin, Sigma) dissolved in EMEM, and NDGA (Sigma) or 5,8,11,14-heneicosatetraenoic acid [5,8,11,14-(21=4)] dissolved in ethanol (3.5×10⁻⁷ M maximum concentration), all sterilized by filtration, were adjusted to the appropriate final concentration in 10% normal fresh serum. The addition of ethanol alone (3.5×10⁻⁷ M) did not affect the cytotoxicity. Platelets were isolated from healthy donors. *B. malayi* larvae were produced *in vitro* as described in Fig. 2. The cytotoxicity assay was performed with 20 µg DEC as in Fig. 1.

the assay. These experiments suggested that, whereas the 'lethal hit' was non-specific, the activation process required a filarial signal. Support for this hypothesis was obtained from experiments in which a filarial excretory antigen (FEA) triggered the cytotoxicity against schistosomula incubated with DEC-treated platelets (Table 1).

In the context of mediator release, it has been claimed that a high concentration of DEC induces the secretion of serotonin by platelets¹³. In our experiments, however, serotonin (from 10⁻¹¹ M to 10⁻⁵ M) was not toxic for parasites and thus could not account for the killing process. Arachidonate metabolites are also formed by activated platelets. A role for the cyclooxygenase pathway was ruled out, as aspirin, an inhibitor of this enzyme, did not modify the platelet activity (Table 2). In contrast, lipoxigenase inhibitors, nordihydroguaiaretic acid (NDGA)¹⁴, esculetin¹⁵ and 5,8,11,14-heneicosatetraenoic acid¹⁶, which also have antioxidant properties, inhibited *B. malayi* killing in a dose-dependent manner (Table 2).

Thus the relationship between parasite killing and the concentration of lipoxigenase inhibitors suggested the production of a cytotoxic lipoxigenase product. Surprisingly, when the lipids of incubates from ¹⁴C-arachidonic acid-prelabelled platelets with DEC plus FEA were extracted at various times, and analysed by thin layer chromatography (TLC), no increase in lipoxigenase, cyclooxygenase or other oxygenated products was noted. To rule out the formation of metabolites at amounts below the sensitivity of TLC, similar studies were carried out

Table 3 Effects of free-radical scavengers and Fe ions, on the DEC-treated platelet activity, and of phenazine methosulphate (PMS) on the platelet cytotoxicity against schistosomula

a, Platelets incubated with 20 µg ml ⁻¹ DEC + 5 µg ml ⁻¹ FEA and with:	% Cytotoxicity against schistosomula (n = 4)
0	92±4
Uric acid 10 ⁻⁴ M	32±2
Sodium formiate 10 ⁻⁴ M	37±3
Mannitol 10 ⁻⁴ M	41±6
Sodium salicylate 3×10 ⁻⁴ M	47±3
Sodium azide 10 ⁻⁴ M	73±5
Catalase 5,000 IU ml ⁻¹	82±5
Superoxide dismutase 1,000 IU ml ⁻¹	93±3
b, Platelets incubated with:	
0	12±4
DEC (5 µg ml ⁻¹ + FEA(1 µg ml ⁻¹)	29±3
DEC (5 µg ml ⁻¹) + FEA(1 µg ml ⁻¹) + Fe ²⁺ (10 ⁻¹¹ M)	73±2
DEC (5 µg ml ⁻¹) + FEA (1 µg ml ⁻¹) + Fe ²⁺ (10 ⁻¹¹ M) + o-phenanthroline 10 ⁻⁶ M	18±5
PMS 10 ⁻⁸ M	73±3
PMS 10 ⁻¹⁰ M	48±4
PMS 10 ⁻¹² M	26±3

The cytotoxic assays were performed with DEC and FEA against *S. mansoni* schistosomula²⁰ using platelets from healthy donors. a, The scavengers of activated oxygen species (sodium formiate, mannitol, uric acid, sodium salicylate, sodium azide), and oxidoreductases (catalase, superoxide dismutase (SOD)) were performed with 20 µg ml⁻¹ DEC and 5 µg ml⁻¹ FEA. b, The action of ferrous sulphate (10⁻¹¹ M), alone or with o-phenanthroline (10⁻⁶ M), was studied in suboptimal conditions of cytotoxicity with 5 µg ml⁻¹ DEC and 1 µg ml⁻¹ FEA. Phenazine methosulphate (PMS) was added to the platelets with the larvae (without DEC or FEA). All the reagents were purchased from Sigma, and were sterilized before being added at the appropriate concentration in the assay. As controls, each reagent was tested without platelets, and gave no direct toxicity. No change was observed in the osmolality and the pH of the medium.

by high pressure liquid chromatography (HPLC) with similar negative results.

Because no lipoxigenase product could be directly implicated in parasite killing, and because the activation of natural killer cells¹⁷ and cytotoxic T lymphocyte-mediated lysis¹⁸ have been similarly examined with the same result, we investigated the possible involvement of oxygen free radicals in platelet-mediated cytotoxicity triggered by DEC as has been proposed for natural killer cell activity^{19,20}. Scavengers of activated oxygen species (sodium formiate, mannitol, uric acid, sodium salicylate and sodium azide) inhibit the platelet killing activity to various degrees (Table 3), but neither catalase nor superoxide dismutase affect the process. A non-specific toxicity of the scavengers on platelets is unlikely; the concentrations do not significantly disturb platelet aggregation and cytotoxicity after the chemicals are washed off. The lack of inhibition of catalase and superoxide dismutase is not due to a failure to reach the site of free-radical formation, as both enzymes block the IgE-dependent cytotoxicity mediated by platelets²¹. Transition metals, particularly iron, are essential for the process leading to lipoperoxidation^{22,23}. Indeed, in suboptimal conditions, Fe²⁺, down to 10⁻¹¹ M enhanced the DEC-induced cytotoxicity of platelets in a dose-dependent manner. Moreover, the Fe²⁺ chelator o-phenanthroline (10⁻⁶ M) abolished the action of iron (Table 3). In addition, phenazine methosulphate (PMS), which is reduced by intracellular NADPH and generates oxygen free radicals by auto-oxidation²⁴, rendered the platelets cytotoxic in the absence of

DEC and FEA. No direct toxicity of, up to 10^{-6} M, PMS on schistosomula was observed.

Further work is required to determine the biochemical mechanisms involved. These data, however, render the participation of (O_x)-type radical species in the microfilicidal properties of DEC-treated platelets highly probable.

We thank H. Vorng for performing the cytotoxicity assays, F. Fouque, D. Henry and D. Monté for technical assistance, J. P. Gazet for maintaining the *B. malayi* cycle and M. F. Massard and C. Colson for secretarial assistance. The 5,8,11,14-heneicosatetraynoic acid was a gift from H. Sprecher (Ohio State University, Columbus, Ohio, USA). We also thank D. Taylor and R. J. Pierce for critical reading of the manuscript. This work was supported in part by funds from the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (ID 860073).

Received 14 November; accepted 25 November 1986.

1. Warren, K. S. & Mahmoud, A. A. *Tropical and Geographical Medicine* 400-401 (McGraw Hill, New York, 1983).
2. Hewitt, R. I. *et al. J. Lab. clin. Med.* **32**, 1314-1329 (1948).
3. Hawking, F. *Adv. Pharmac. Chemother.* **16**, 129-194 (1979).

4. Hawking, F., Sewell, P. & Thurston, J. P. *Brit. J. Pharmac.* **5**, 217-238 (1950).
5. Piessens, W. F. & Beldekas, N. *Nature* **282**, 845-847 (1979).
6. Mustard, J. F., Perry, D. W., Ardlie, N. G. & Patcham Brit. *J. Haematol.* **22**, 193-204 (1972).
7. Patscheke, H. & Wörner, P. *Thromb. Res.* **12**, 485-496 (1978).
8. Muylle, L., Taelman, H., Moldenhauer, R., VanBrabant, R. & Peetermans, M. *Br. med. J.* **287**, 519-520 (1983).
9. Haque, A., Cuna, W., Bonnel, B., Capron, A. & Joseph, M. *Parasite Immun.* **7**, 517-525 (1985).
10. Ash, L. R. *J. Parasit.* **59**, 442-447 (1973).
11. Stanionas, R. J. & Hammerberg, B. *J. Parasit.* **68**, 809-816 (1982).
12. Kobayashi, J., Matsuda, H., Fujita, K., Sakai, T. & Shinoda, K. *Jap. Parasit.* **18**, 563-574 (1969).
13. Kowalski, K. A., McConnel, L. A., Sadoff, D. A. & Weskeid, R. *Am. J. Pathol.* **113**, 1-7 (1983).
14. Tappel, A. L., Lundberg, W. O. & Boyer, P. D. *Arch. Biochem. Biophys.* **42**, 293-304 (1953).
15. Sekiya, K., Okuda, H. & Arichi, S. *Biochem. biophys. Acta* **713**, 68-72 (1982).
16. Wilhelm, T. E., Sankarappa, S. K., van Rollins, M. & Sprecher, H. *Prostaglandins* **21**, Vol. 2, 323-332 (1981).
17. Bray, R. A. & Brahm, Z. *J. Immun.* **136**, 1783-1790 (1986).
18. Taylor, A. S., Howe, R. C., Morrison, A. R., Sprecher, H. & Russell, J. H. *J. Immun.* **134**, 1130-1135 (1985).
19. Suthanthiran, M. *et al. Nature* **307**, 276-278 (1984).
20. Duwe, A. K., Werkmeister, J., Roder, J. C., Lauzon, R. & Payne, U. *J. Immun.* **134**, 2637-2644 (1985).
21. Joseph, M. *et al. Eur. J. Immun.* **16**, 306-312 (1986).
22. Heaton, F. H. & Uri, N. *J. Lipid. Res.* **2**, 152-160 (1961).
23. Aust, S. D. & Svenger, B. A. in *Free Radicals in Biology* Vol. 5, (ed. Pryor, W. A.) 1-28 (Academic, New York, 1984).
24. Mishikimi, M., Rao, N. A. & Yagi, K. *Biochem. biophys. Res. Commun.* **46**, 849-854 (1972).
25. Caen, J. P., Nurdan, A. T. & Jeanneau, C. *J. Lab. clin. Med.* **87**, 586-596 (1976).
26. Aggarwal, A., Cuna, W., Haque, A., Dissous, C. & Capron, A. *Immunology* **54**, 655-663 (1985).

Role of *Staphylococcus* protease in the development of influenza pneumonia

M. Tashiro*, P. Ciborowski†, H.-D. Klenk*, G. Pulverer† & R. Rott*

* Institut für Virologie, Justus-Liebig-Universität Giessen, 6300 Giessen, FRG

† Institut für Hygiene, Universität Köln, 5000 Köln, FRG

In influenza the combined virus-bacterial pneumonia is approximately three times more common than primary viral pneumonia¹. The bacteria most commonly involved are *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Haemophilus influenzae*. *S. aureus* co-infection is reported to have a fatality rate of up to 42% (ref. 2). It is thought that virus infection in the respiratory tract favours growth conditions for bacteria. In this letter data are presented which show that some *S. aureus* strains secrete a protease which exerts a decisive influence on the outcome of influenza virus infection in mice by cleavage activation of the virus haemagglutinin.

In a host cell where an appropriate protease for post-translational proteolytic cleavage of the precursor haemagglutinin (HA) of influenza virus into the HA₁ and HA₂ fragments is not present and as a consequence infectious virus cannot be produced, infectivity can be recovered by *in vitro* treatment of non-infectious virus by trypsin^{3,4}. We have found that by analogy a trypsin-like protease isolated from cell-free culture medium of some *S. aureus* strains can activate *in vitro* infectivity of a number of influenza virus strains. All mammalian and most of the avian influenza virus strains are produced in chicken embryo cells (CEC) in non-infectious form⁵. If such virus particles were treated *in vitro* with *S. aureus* protease, the infectivity of most strains was enhanced at least 100-fold. The increase in infectivity by treatment with *S. aureus* protease and trypsin was similar, although, in contrast to the bacterial enzyme, trypsin activated the virus of all strains tested. Furthermore, in CEC infected with strains which could be activated by *S. aureus* protease, the presence of the bacterial enzyme in the culture medium enabled the virus to undergo multiple growth cycles.

Polyacrylamide gel electrophoresis (PAGE) revealed that activation of virus infectivity was paralleled by cleavage of HA as shown for the virus strain A/swine/1976/31 (H1N1) in Fig. 1. The cleavage products of HA, HA₁ and HA₂, obtained after

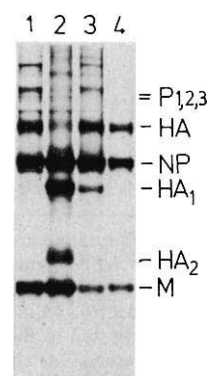


Fig. 1. SDS-PAGE of proteins of A/swine/1976/31 (H1N1) virus grown in chicken embryo cells, labelled with ³⁵S-methionine 5 h after infection. Virus was isolated from culture medium 18 h after infection, purified by ultracentrifugation, and treated for 30 min at 37 °C with 10 µg trypsin (lane 2), 250 µg *S. aureus* Wood 46 protease (lane 3) or 250 µg (*S. aureus* M/86/86 protease (lane 4) per ml before gel electrophoresis. Lane 1 not treated. P_{1,2,3}, polymerase proteins PB₁, PB₂, PB₃; NP, nucleoprotein; M, membrane protein.

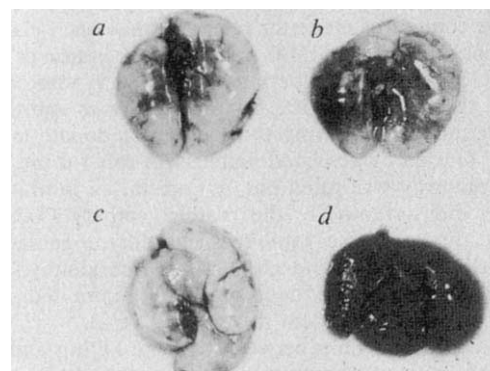


Fig. 2. Pathological alterations in the lungs of mice a, mock infected; b, intranasally infected with A/swine/1976/31 (H1N1) virus; c, infected with *S. aureus* strain Wood 46 and d, co-infected with H1N1 virus and *S. aureus* Wood 46, respectively. The lungs were taken 5 days after infection. Doses of inoculum were 10^2 plaque-forming units (p.f.u.) of virus and 10^6 colony-forming units (c.f.u.) of bacterium.

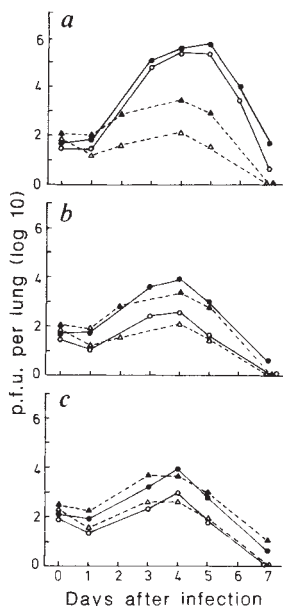


Fig. 3. Virus titres obtained from lung homogenates of mice intranasally infected with 10^2 p.f.u. of influenza virus and 10^6 c.f.u. of *S. aureus*. Tissue samples were either treated with trypsin ($10 \mu\text{g ml}^{-1}$) for 15 min at 37°C or not treated before the plaque test⁵. *a*, Co-infection with A/swine/1976/31 (H1N1) virus and *S. aureus* Wood 46; *b*, co-infection with A/chicken/Germany/49 (H10N7) virus and *S. aureus* Wood 46; *c*, co-infection with H1N1 virus and *S. aureus* M/86/86. Infections with virus alone and treatment Δ , without or $\blacktriangle$, with trypsin; combined infections $\circ$, without or $\bullet$, with trypsin.

treatment with the protease isolated from the *S. aureus* strain Wood 46 (ATCC/Nr. 10832) migrated to the same position as those obtained after trypsin treatment. On the other hand, the HA of virus strains which were not activated by the bacterial enzyme, such as A/chick/Germany/49 (H10N7) was found to be uncleaved after protease treatment.

Not all of the *S. aureus* strains tested secrete a protease that activates influenza virus. The highest enzymatic activity was found with the strain Wood/46 whereas, for instance, the mouse isolate M/86/86 did not produce an enzyme suitable for HA cleavage of several influenza virus strains.

A combined virus-bacterial infection was performed in mice using the influenza virus A/swine/1976/31 (H1N1) and the *S. aureus* strain Wood/46. Intranasal co-infection resulted in a fatal disease with extended lesions in the lung tissue (Fig. 2). The animals usually died within 5 days of infection. In contrast, after intranasal infection with virus or bacteria alone, the animals did not produce significant symptoms or relevant pathological changes in the lung during an observation period of 7 days. As shown in Fig. 3, only low virus titres were found in lung homogenates of mice infected with virus alone. But the infectivity of such tissue samples was greatly enhanced by adding trypsin before the plaque test⁵. Thus, after infection with virus alone a substantial amount of virus was produced in the mouse lung, most of which was non-infectious. In contrast, after co-infection with *S. aureus*, high titres of infectious virus were produced in the lung, but the titre was not increased by *in vitro* treatment with trypsin. These data indicate that the drastically enhanced pathogenicity observed after co-infection was due to virus activation by the *S. aureus* protease. Two controls underlined the role of the *S. aureus* enzyme in the outcome of the disease: co-infection of swine influenza virus with the *S. aureus* strain M/86/86, which lacks an appropriate protease, did not result in disease. Neither did co-infection of the protease-producing strain Wood 46 and the virus strain A/chicken/Germany/49, which has an HA insensitive to *S. aureus* protease activation,

lead to significant signs of a disease. In both cases most of the virus produced in the mice lung was non-infectious, as was also observed when the mice were infected with the respective viruses alone.

These results suggest that the decisive mechanism responsible for the development of influenza pneumonia is the ability of the virus to produce large amounts of progeny in multiple replication cycles, allowing extensive spread of infection in the lung before the host defence becomes effective. Usually influenza virus infection does not accomplish this, because the large majority of the virus particles produced in the lung are non-infectious. But micro-organisms that are relatively harmless by themselves may release the block in virus multiplication through proteolytic activation of the haemagglutinin. The data obtained again underline the role of the haemagglutinin as a determinant for influenza virus pathogenicity⁶ and may explain on a molecular level the synergistic effect between influenza viruses and ubiquitous micro-organisms for the development of fatal pneumonia in man and animals.

We thank Dr M. Reinacher for the pathological examinations. The work was supported by the Deutsche Forschungsgemeinschaft (SFB 47) and the Fonds der Chemie. M.T. and P.C. were recipients of a research fellowship of the Alexander von Humboldt-Foundation.

Received 26 August; accepted 8 December 1986.

1. Stuart-Harris, C. H. *Am. Rev. resp. Dis.* **83**, 54-67 (1961).
2. Robertson, L., Caley, J. P. & Moore, J. *Lancet* **ii**, 233-236 (1958).
3. Klenk, H.-D., Rott, R., Orlich, M. & Blödmann, J. *Virology* **68**, 426-439 (1975).
4. Lazarowitz, S. G. & Choppin, P. W. *Virology* **68**, 440-454 (1975).
5. Zhirnov, O. P., Ovcharenko, A. V. & Burkinskaya, A. G. *Arch. Virol.* **71**, 177-183 (1982).
6. Rott, R. & Klenk, H.-D. in *Options for the Control of Influenza* (eds Kendal, A. P. & Patriarca, P. A.) 53-62 (Liss, New York, 1986).

Receptors for B-cell stimulatory factor-1 expressed on cells of haematopoietic lineage

Junichi Ohara & William E. Paul

Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA

B-cell stimulatory factor-1 (BSF-1) is a T-cell product of relative molecular mass 20,000 (M_r 20K) initially described as a cofactor required for DNA synthesis by resting mouse B cells stimulated with low concentrations of anti-IgM antibodies¹. It acts on resting B cells to enhance the expression of class II major histocompatibility complex (MHC) molecules^{2,3}, to prepare these cells to respond more promptly to subsequent stimuli, such as anti-IgM antibodies^{4,5}, and causes the secretion of IgG₁ and IgE by B cells stimulated with lipopolysaccharide (LPS)⁶⁻⁹. BSF-1 has been shown to stimulate T cell lines¹⁰, resting T cells¹¹ and some mast cell lines¹⁰. Recently, the designation interleukin-4 (IL-4) has been suggested for BSF-1. We report here the existence of high-affinity cell-surface receptors specific for BSF-1 on both B and T lymphocytes, and on cells of several other haematopoietic lineages, including mast cell, macrophage and undifferentiated haematopoietic cell lines. Resting B and T lymphocytes express receptors, which increase in number upon activation of B cells with LPS or anti-IgM, and of T cells with concanavalin A. Cross-linking of ¹²⁵I-labelled-BSF-1 to its receptors creates a complex of M_r ~80,000.

BSF-1 has been biochemically characterized and purified to homogeneity (refs 12, 13; Ohara, *et al.*, in preparation). The N-terminal 24 amino acids have a sequence which matches that deduced from the nucleotide sequence of BSF-1 complementary

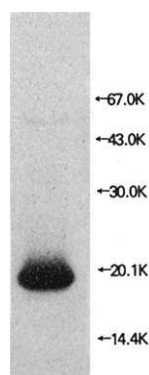


Fig. 1 ^{125}I -labelled BSF-1. Purified BSF-1 was obtained from supernatant fluids of 4- β -phorbol-12 β -myristate-12 α -acetate (PMA)-activated EL-4 cells by a combination of affinity chromatography and HPLC. Twelve litres of EL-4 supernatant was passed over an affinity column of 10 ml Sepharose-4B conjugated with 20 mg γ -globulin from a monoclonal anti-BSF-1 (11B11) ascites, at a flow rate of 4 ml min $^{-1}$. After washing the column thoroughly with 2 l glass-distilled water, BSF-1 was eluted with 100 ml 0.1% trifluoroacetic acid (TFA), which was immediately passed through a 0.45 μm filter and loaded onto a C18-reversed phased HPLC column (5 μm particle size, $\frac{1}{4} \times 25$ cm, Cat. no. 88,984, Supelco, Inc.) with a computed HPLC system (Perkin-Elmer Corp.) as described¹². The column was developed with a linear gradient of 35–55% acetonitrile in 0.1% TFA over 60 min at a flow rate of 0.5 ml min $^{-1}$. Homogenous BSF-1 emerged in both the 41.0% and 45.0% acetonitrile fractions. The two fractions were combined and twice lyophilized. Purified BSF-1 (20 μg), 2.5 mCi Na ^{125}I (Amersham; 1,800 Ci mmol $^{-1}$) and an Enzymobeads suspension (Bio-Rad Lab, Richmond, CA) in 78.5 μl of 0.1 M sodium phosphate buffer (pH 7.0) was placed in a 1.5-ml polypropylene tube. The iodination reaction was started by adding 9 μl of 6% D-glucose solution (Fisher Scientific) and continued for 16 h at 4°C. The reaction was stopped by adding 25 μl of 10% sodium azide in 0.1 M sodium phosphate buffer (pH 7.0), incubating for 15 min on ice, followed by the addition of 200 μg tyrosine. The mixture was applied to a Sephadex G-25 column (0.5 $\times$ 10 cm), which had been equilibrated with phosphate buffered saline containing bovine serum albumin at 0.1%. Separation of bound and free iodine was done by a step-by-step fractionation with 100 μl aliquots of the same buffer. ^{125}I -labelled BSF-1 (10,000 c.p.m.) was mixed with 20 μg of low molecular weight calibration proteins (Pharmacia) and analysed by 20% SDS-PAGE and autoradiography.

DNA clones^{14,15}. Both monoclonal and rabbit anti-peptide antibodies specific for BSF-1 have been prepared (ref. 16; Ohara *et al.*, in preparation).

We purified BSF-1 from supernatant fluids of phorbol myristate acetate (PMA)-stimulated EL-4 cells by affinity chromatography using a Sepharose 4B-monoclonal anti-BSF-1 antibody (11B11) column. Eluted material was placed on a C18 reverse-phase high-pressure liquid chromatography (HPLC) column developed with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid (TFA). Pure material was obtained in two fractions (41 and 45% acetonitrile, respectively). This material was radioiodinated with Enzymobeads (Fig. 1); the resultant ^{125}I -labelled-BSF-1 bound to spleen cells from DBA/2 mice in a saturable manner (Fig. 2a), reaching maximum binding at both 4°C and 37°C within 30 minutes. This binding was strikingly inhibited with a 100–200 fold molar excess of purified non-radioactive BSF-1; recombinant human IL-2, at a concentration of 4.1×10^5 units ml $^{-1}$, had no inhibitory effect on binding of ^{125}I -labelled-BSF-1.

A Scatchard analysis of the binding of ^{125}I -labelled-BSF-1 to DBA/2 spleen cells (Fig. 2b) indicated the existence of a class of high affinity receptor sites; a mean equilibrium constant for binding to DBA/2 spleen cells of $3.3 \times 10^{10} \text{ M}^{-1}$ was obtained

Table 1 BSF-1 receptor expression on various cell types

	Cell type	Receptors per cell (mean $\pm$ s.e.)	K (M^{-1})
Spleen cell	DBA-2	450 $\pm$ 38*	3.3 $\pm$ 0.7 $\times 10^{10}\dagger$
B cell	Resting	311 $\pm$ 23	
	LPS blast	1511 $\pm$ 331	
	Monoclonal anti-IgM blast	1,560	
	R8/205	810	
	BAL 17	420	
T cell	Resting	280	
	Con A blast	2,420	
	CTLL	830	
	HT-2	2,085	
Macrophage	P388D1	1,104	
	IC21	2,012	
Mast cell	ABFTL-2	2,170	1.8 $\times 10^{10}$
	MMC1	2,720	
	MMC34	3,630	
	MC4	2,520	
	P815	1,290	
Other haematopoietic	DA-1	670	
Human	T cell	Undetectable	
	Jurkat	Undetectable	
	B cell	Undetectable	
	Raji	Undetectable	
	U937	Undetectable	
	HeLa	Undetectable	

* Number of replicates (n): DBA/2 spleen cells; 10; resting B cells; 3; LPS blasts; 4; Con A blasts; 2; all others; 1.

$\dagger n = 3$.

Table 2 IFN γ , anti-Lyb-2.1 and anti-LFA-1 fail to block binding of ^{125}I -labelled BSF-1 to spleen cells

Exp.	Cell type	Inhibitor	^{125}I -labelled BSF-1 bound (counts)
1	DBA/2 B cells	None	1,398
		BSF-1 (50 ng ml $^{-1}$)	471
		Anti-Lyb 2.1 (50 μg ml $^{-1}$)	1,406
2	DBA/2 spleen cells	None	1,819
		BSF-1 (50 ng ml $^{-1}$)	422
		IFN γ (2.5 μg ml $^{-1}$)	1,713
		Rat IgG ((60 μg ml $^{-1}$)	1,788
		Anti-BSF-1 (10 μg ml $^{-1}$)	524
		Anti-LFA-1 (50 μg ml $^{-1}$)	
		M18/2.a.8	1,771
		M17/4.2	1,745
		FD441.8	1,773

Experiment 1: 1.5×10^7 DBA/2 (Lyb-2.1 $^{+}$) resting splenic B cells were incubated with or without BSF-1 or anti-Lyb-2.1; ^{125}I -labelled BSF-1 was added to a final volume of 410 μl binding buffer and cells were incubated for 30 min at room temperature. Three 130- μl cell aliquots were layered onto 200 μl mixed oil. In experiment 2, 1.6×10^7 DBA/2 spleen cells were incubated for 15 min at 4°C in 410 μl binding buffer containing 20 mM 2-deoxy-D-glucose, with or without BSF-1, various antibodies, or recombinant mouse IFN γ (Genentech, Inc.). Three 100- μl cell aliquots were layered onto 200 μl mixed oil.

from three independent analyses. From 10 separate measurements, we estimate that DBA/2 spleen cells had 450 receptors per cell (Table 1). Resting splenic B cells and mesenteric lymph node T cells were prepared as previously described^{4,11}; both cell types have mean cell volumes of $\sim 100 \mu\text{m}^3$. These cells possessed 310 and 280 receptors per cell, respectively. Activation of B cells by stimulation with a monoclonal anti-IgM antibody and with LPS caused a substantial increase in receptor number. Similarly, activation of T cells with concanavalin A for 48 h

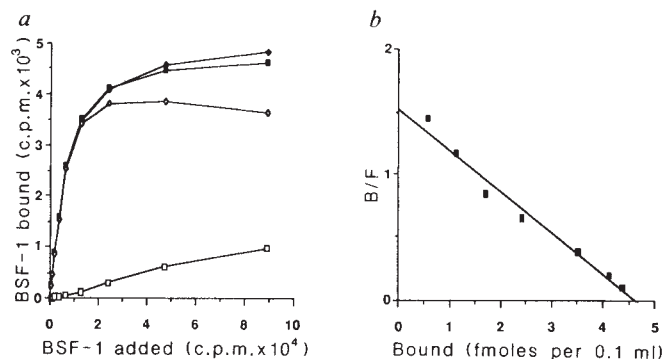


Fig. 2 *a*, Binding of ¹²⁵I-labelled BSF-1 to DBA/2 spleen cells. ■, total bound; ◆, IL-2 inhibited; □, BSF-1 inhibited; ◇, specific bound; *b*, Scatchard plot of BSF-1 binding to spleen cells; $y = 1.53 - 0.33x$; $R = 0.99$; $K = 3.31 \pm 0.81 \times 10^{10}$ L/M; 475 receptors per cell. Thrice-washed DBA/2 spleen cells (1.6×10^7), and ¹²⁵I-labelled BSF-1 were incubated in 410 μ l of RPMI 1640 medium containing 10% FCS, 0.2% sodium azide, 20 mM HEPES (pH 7.0). In some tubes a 100–200-fold molar excess of purified non-radioactive BSF-1 or recombinant IL-2 at a maximal concentration of 4.1×10^5 units per ml was present. The reaction mixture was incubated at 4 °C for 2 h; during the incubation period, the tubes were shaken vigorously on an orbital shaker and mixed with a vortex mixer at 15 min intervals. Three 100 μ l aliquots were separated into bound and free ¹²⁵I-labelled BSF-1 achieved by centrifugation through 200 μ l of a mixture of silicon oil DC550 (Serva) and white light paraffin oil (Fisher Scientific) at a ratio of 84 to 16. After centrifugation for 5 min at the highest speed of Beckman Microfuge-12, the tubes (Bel-Art Products) were frozen in dry ice and the cell pellets were separated from the supernatant by slicing the tube. The radioactivities of both cell pellets and supernatants were counted with Model gamma 5,500 γ -counter (Beckman Instruments) and were used as a BSF-1 receptor associated and nonassociated counts, respectively. Experiments in *a* and *b* were carried out with separate preparations of ¹²⁵I-labelled BSF-1 and with separate spleen cell preparations. In our measurement of specific activity, we assumed that all the ¹²⁵I-labelled BSF-1 was active. We compared the diminution in binding of a standard amount of ¹²⁵I-labelled BSF-1 as increasing amounts of ¹²⁵I-labelled BSF-1 were added with the diminution in binding that occurred with the addition of increasing amounts of purified BSF-1 whose concentration had been determined by amino-acid analysis. Based on this, the specific activity of the ¹²⁵I-labelled BSF-1 used in the Scatchard analysis (Fig. 2*b*) was 7.05×10^{-18} mmol per c.p.m. Although the linearity of the Scatchard analysis ($R = 0.99$) is consistent with the assumption that all ¹²⁵I-labelled BSF-1 was active, only 60% of the radioactivity bound to cellular receptors at the lowest ligand concentration used in the experiment shown in Fig. 2*b*. As free BSF-1 must also be present at equilibrium, a minimum estimate for the activity of ¹²⁵I-labelled BSF-1 is ~70%. Recalculated values for K and receptor number assuming that only 70% of ¹²⁵I-labelled BSF-1 was active are 4.5×10^{10} M⁻¹ and 650 receptors per cell, respectively. These values are only modestly higher than those obtained assuming all labelled BSF-1 is active. For this reason, all subsequent values for K and receptor number are based on the assumption of 100% active ¹²⁵I-labelled BSF-1.

caused a striking enhancement in receptor expression (Table 1). Lines of B-cell origin (R8.205, an Abelson virus transformed pre-B-cell line, and BAL-17, a mature B lymphoma) expressed receptors, as did the IL-2-dependent T cell lines HT-2 and CTLL. HT-2 has been shown to be more responsive to BSF-1 than CTLL (unpublished observation). In this respect, the finding that HT-2 cells had more receptors for BSF-1 than CTLL cells may be important (Table 1). A series of 5 factor-independent long-term mast cell lines^{17,18} all proved to have relatively large numbers of receptors. Scatchard analysis of the binding of ¹²⁵I-labelled BSF-1 to the mast cell line ABFTL-2 yielded an equilibrium constant of 1.8×10^{10} M⁻¹, which is very similar to that of DBA/2 spleen cells (Table 1). The macrophage lines,

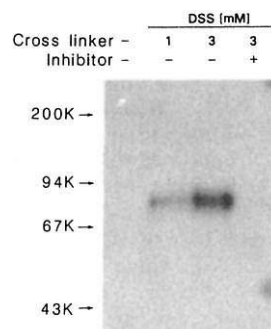


Fig. 3 Cross-linkage of ¹²⁵I-labelled BSF-1 to DBA/2 spleen cell surface molecules. DBA/2 spleen cells (1.5×10^8) were mixed with 3.3 ng ¹²⁵I-labelled BSF-1 in 550 μ l HBSS containing 0.2% sodium azide and 1 mM phenylmethyl sulphonyl fluoride (P-7626, Sigma) and incubated for 30 min at 4 °C. After washing the cells once with 8 ml cold buffer, they were resuspended in 360 μ l cold buffer and 40 μ l of 10 mM or 30 mM disuccinimidyl suberate (DSS Pierce Chemical Co.), dissolved in dimethylsulphoxide (Fisher Scientific), was added. DMSO (40 μ l) was used in a negative control experiment and 200-fold excess purified nonradioactive BSF-1 was added, together with 3 mM DSS in order to study the specificity of ¹²⁵I-labelled BSF-1-receptor complex cross-linkage. The cross-linkage reaction was accomplished by 20 min incubation at 4 °C and was stopped by adding 100 μ l 1 M ammonium acetate. After 5 min incubation at 4 °C, the cells were washed once with 4 ml cold HBSS. Then the cells were lysed at 4 °C in 0.4 ml lysis buffer consisting of 10% glycerol, 2% SDS, 2% β -mercaptoethanol, 0.01% bromophenol blue. The lysate was boiled for 5 min and centrifuged for 2 h at 35,000 r.p.m. at 20 °C in order to obtain a clear lysate. The supernatant was removed and 50- μ l aliquots were subjected to electrophoresis on 10% polyacrylamide gels in 0.1% SDS and analysed by autoradiography.

IC21 and P388D1 and the IL-3 dependent haematopoietic cell line DA-1¹⁷ also expressed BSF-1 receptors. The ¹²⁵I-labelled BSF-1 failed to bind to normal human peripheral blood B and T cells and to human B-cell, T-cell and macrophage lines, suggesting that the binding of BSF-1 to its receptor is species-specific (Table 1).

The binding of ¹²⁵I-labelled BSF-1 to spleen cells was inhibited by excess non-radioactive BSF-1 but not by recombinant IL-2 (Fig. 2*a*) or by recombinant mouse IFN γ (Table 2). The latter result is important because IFN γ blocks the effects of BSF-1 on resting B cells¹⁹. Monoclonal anti-BSF-1 antibody blocks the binding of ¹²⁵I-labelled BSF-1 to the same extent as non-radioactive BSF-1 (Table 2). Recently, it has been suggested that Lyb-2 or LFA-1 might be membrane receptors for BSF-1^{20,21}. However, one monoclonal anti-Lyb-2.1 antibody and three monoclonal anti-LFA-1 antibodies (including two specific for the LFA-1 α -chain) failed to inhibit binding of ¹²⁵I-labelled BSF-1 even at concentrations of 50 μ g ml⁻¹. The failure of anti-Lyb-2.1 to block binding, together with the finding that Lyb-2 negative cells (T cells) express BSF-1 receptors, strongly suggests that Lyb-2 is not the receptor for BSF-1. The failure of anti-LFA-1 to inhibit binding also argues against its role as a receptor for BSF-1; furthermore, Glimcher *et al.* have shown that R8.205, which expresses BSF-1 receptors, and responds to BSF-1²² is LFA-1⁻ (personal communication). This makes it unlikely that LFA-1 is the BSF-1 receptor. The possibility that either or both antigens are part of receptor complex is not excluded.

DBA/2 spleen cells were incubated with ¹²⁵I-labelled BSF-1, washed and then treated with the bifunctional cross-linking agent disuccinimidyl suberate (DSS), according to methods used to characterize the gonadotropin receptor²³ (Fig. 3). A new radioactive species with M_r ~80K by sodium dodecyl sulphate-polyacrylamide gel electrophoresis was observed, presumably

consisting of a complex of ^{125}I -labelled BSF-1 and a polypeptide chain of its receptor. The complex did not form in the absence of DSS, and was not detected in the presence of a large excess of non-radioactive BSF-1. A similar radioactive band was observed when BSF-1 was cross-linked on the mast cell line ABFTL-2, but no such band was observed with receptor-negative human HUT-102R cells. From these results, we tentatively conclude that the BSF-1 receptor of spleen cells and mast cells consists of or contains a chain of $M_r \sim 60\text{K}$. This further supports the possibility that LFA-1, which contains α and β chains of 180,000 and 95,000, respectively²⁴, is not the receptor for BSF-1.

These results demonstrate a high-affinity BSF-1-binding molecule on the surface of many of the cell types known to be sensitive to the action of BSF-1. These include B cells, T cells, mast cells, macrophages, and undifferentiated haematopoietic cells. Recently, BSF-1 has also been shown to co-stimulate the appearance of granulocytic megakaryocytic and erythroid colonies in soft agar seeded with anti-Thy-1-treated mouse bone marrow²⁵ suggesting that BSF-1 receptors will be found on all haematopoietic lineage cells.

The expression of BSF-1 receptors on resting B and T cells, in contrast to the lack or very limited expression of IL-2 receptors on such cells⁶, is consistent with the demonstration that resting lymphocytes are a major target of BSF-1 activity²⁻⁵. We have detected only a single class of high-affinity receptor sites; considering the concentrations of BSF-1 which we used in these binding studies (maximum concentrations $1.5 \times 10^{-8}\text{ M}$), we cannot exclude the existence of a second class of receptor sites of lower affinity. The chemical and antigenic characterization of the BSF-1 receptor is now a major goal which should be markedly aided by the development of this binding assay.

We thank Dr W. Lee Maloy for carrying out amino-acid analyses, Drs James Ihle and Reuben Seraganian for providing us with long term mast cell lines, Drs J. Hwang, T. Okuno, T. Nakagawa and N. Nakagawa for helpful suggestions and advice, William Gruen, Jane Hu-Li and Cynthia Watson for technical assistance and Shirley Starnes for the preparation of this manuscript.

Received 20 October; accepted 10 December 1986.

- Howard, M. *et al.* *J. exp. Med.* **155**, 914-923 (1982).
- Roehm, N. W. *et al.* *J. exp. Med.* **160**, 679-694 (1984).
- Noelle, R., Krammer, P. H., Ohara, J., Uhr, J. W. & Vitetta, E. S. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6149-6153 (1984).
- Rabin, E. M., Ohara, J. & Paul, W. E. *Proc. natn. Acad. Sci. U.S.A.* **82**, 2935-2939 (1985).
- Oliver, K., Noelle, R. J., Uhr, J. W., Krammer, P. H. & Vitetta, E. S. *Proc. natn. Acad. Sci. U.S.A.* **82**, 2465-2467 (1985).
- Vitetta, E. S. *et al.* *Immun. Rev.* **78**, 137-157 (1984).
- Sideras, P., Bergstedt-Lindqvist, S. & Severinson, E. *Eur. J. Immun.* **15**, 593-598 (1985).
- Vitetta, E. S. *et al.* *J. exp. Med.* **162**, 1726-1731 (1985).
- Coffman, R. L. *et al.* *J. Immun.* **136**, 4538-4541 (1986).
- Mosmann, T. R., Bond, M. W., Coffman, R. L., Ohara, J. & Paul, W. E. *Proc. natn. Acad. Sci. U.S.A.* **83**, 5654-5658 (1986).
- Hu-Li, J. *et al.* *J. exp. Med.* **165**, 157-172 (1987).
- Ohara, J., Lahet, S., Inman, J. & Paul, W. E. *J. Immun.* **135**, 2518-2523 (1985).
- Grabstein, K. *et al.* *J. exp. Med.* **163**, 1405-1414 (1986).
- Noma, Y. *et al.* *Nature* **319**, 640-646 (1986).
- Lee, F. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **83**, 2061-2065 (1986).
- Ohara, J. & Paul, W. E. *Nature* **315**, 333-336 (1985).
- Pierce, J. H. *et al.* *Cell* **41**, 685-693 (1985).
- Barsumian, E. L., McGivney, A., Basciano, L. & Siraganian, R. *Cell. Immun.* **90**, 131-141 (1985).
- Rabin, E. M., Mond, J. J., Ohara, J. & Paul, W. E. *J. Immun.* **137**, 1573-1576 (1986).
- Yakura, H., Kawabata, I., Shen, F. W. & Katagiri, M. *Fedn Proc.* **44**, 1532 (1985).
- Mishra, G. C. *et al.* *J. Immun.* **137**, 1590-1598 (1986).
- Polla, B. S. *Proc. natn. Acad. Sci. U.S.A.* **83**, 4878-4882 (1986).
- Hwang, J. & Menon, K. M. J. *J. biol. Chem.* **259**, 1978-1985 (1984).
- Kurzinger, K. *et al.* *J. Immun.* **127**, 596-602 (1981).
- Peschell, C. *et al.* *Blood* (submitted).
- Robb, R. J., Munck, A. & Smith, K. A. *J. exp. Med.* **154**, 1455-1474 (1981).

Association of phosphorylation of the T3 antigen with immune activation of T lymphocytes

D. Cantrell*, A. A. Davies*, M. Londeit*, M. Feldman† & M. J. Crumpton*

* Cell Surface Biochemistry, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, UK
† Charing Cross Sunley Research Centre, Lurgan Avenue, London W6, UK

In human T lymphocytes the antigen receptor (Ti) is associated non-covalently on the cell surface with the invariant T3 antigen which comprises 3 chains: two glycosylated polypeptides of relative molecular mass 26,000 (M_r 26K) and 21K (γ and δ) and one non-N-glycosylated polypeptide of M_r 19K (ϵ)¹⁻⁷. The proposed function of T3 is to transduce the activation signals delivered via the antigen receptor⁸⁻¹³. Recently we have shown that phorbol esters, which stimulate protein kinase C, can induce phosphorylation of the γ subunit of the T3 antigen^{14,15}. But the critical question is whether T3 phosphorylation occurs as a normal consequence of immune activation of T lymphocytes. In this respect, it has been shown that immune stimulation of murine T cells results in phosphorylation of Ti-associated polypeptides that may be the functional analogues of the human T3 antigen^{16,17}. We have therefore monitored T3 phosphorylation after exposure of human T cells to antigen or phytohaemagglutinin (PHA). The data show that both stimuli initiate phosphorylation of the γ subunit of the T3 antigen which indicates that T3 phosphorylation is a physiological response to immune activation.

To explore whether T3 phosphorylation accompanies polyclonal T-cell activation, T3 immunoprecipitates were prepared from ^{32}P -labelled quiescent peripheral blood T cells that had been exposed to the lectin PHA. There were no T3 phosphory-

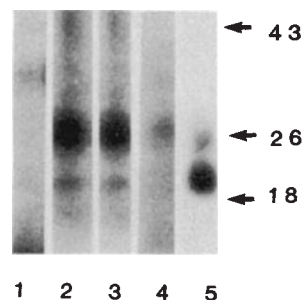


Fig. 1 The ^{32}P -labelled peripheral blood derived lymphocytes were treated with PHA for 0, 5, 15, 40 min (tracks 1, 2, 3, 4 respectively) before immunoprecipitation with UCHT1 (a monoclonal antibody against the T3 antigen). Track 5 shows a control immunoprecipitation of T3 antigen prepared from surface ^{125}I -labelled T lymphoblasts.

Methods. Human peripheral blood mononuclear cells from a single donor were isolated by Ficoll-Hypaque discontinuous gradient centrifugation and cultured (10^6 per ml) for 18 h in phosphate-free Eagle's medium supplemented with 5% heat-inactivated dialysed fetal calf serum and $40\text{ }\mu\text{Ci ml}^{-1}$ of (^{32}P)-orthophosphate (Amersham) before addition of $2\text{ }\mu\text{g ml}^{-1}$ of PHA (Burroughs Wellcome). Cell lysis and immunoprecipitation were performed as described¹⁵. Briefly, 2×10^7 cells were extracted with 1 ml of lysis buffer (1% Nonidet P40 in 10 mM Tris/HCl buffer, pH 7.4, containing 0.15 M NaCl, 1% BSA, 1 mM phenylmethanesulphonyl fluoride, 1 mM EDTA and 50 mM NaF) for 10 min at 4°C . After centrifuging for 195,000g min, lysates were pre-cleared with fixed *Staphylococcus aureus* organisms and rabbit anti-mouse immunoglobulin. Pre-cleared lysate (1 ml) was precipitated with 5-10 μg of monoclonal antibody UCHT1¹⁸, covalently coupled to Sepharose 4B beads (Pharmacia Fine Chemicals). Immunoprecipitates were washed sequentially with lysis buffer containing: 0.65 M NaCl (1); lysis buffer plus 0.1% SDS (2); 0.1% Nonidet P40 in 10 mM Tris/HCl, pH 7.4 (3), and then analysed by SDS-PAGE on a 12% gel run under reducing conditions. Cells were surface labelled with ^{125}I by lactoperoxidase catalysed iodination as described¹⁹.

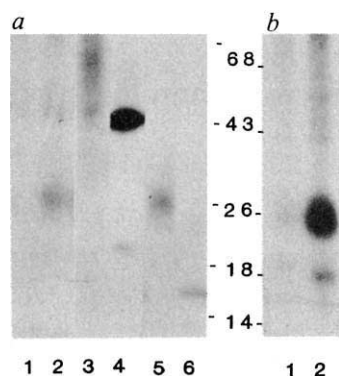


Fig. 2 *a*, Analyses of immunoprecipitates prepared from ^{32}P -labelled peripheral-blood-derived T cell blasts which had been treated with $2\ \mu\text{g ml}^{-1}$ of PHA for 0 (track 1) or 5 min (tracks 2–6). Tracks 1, 2, 5, 6 are T3 immunoprecipitates prepared using the monoclonal antibody UCHT1, described in the legend to Fig. 1. Tracks 3 and 4 shows immunoprecipitates of T11 and HLA-A, B, C, antigens prepared using the monoclonal antibodies RFT11 and W6/32 respectively²⁰. Track 6 shows a T3 immunoprecipitate prepared from PHA activated T cells and then digested with endo- β -N acetylglucosylaminidase F as described¹⁴. *b*, Analyses of T3 immunoprecipitates prepared from ^{32}P -labelled T lymphoblasts which had been activated with $2\ \mu\text{g ml}^{-1}$ of PHA for 5 min (track 1) or $50\ \text{ng ml}^{-1}$ of Pdbu for 5 min. The relative intensities of the T3 γ -chain labelling in PHA and phorbol ester treated cells were compared by scanning autoradiographs densitometrically using a Joyce-Loebl Chromoscan 3. The relative intensities of the bands were compared on the basis of peak areas (arbitrary units).

Methods. T lymphoblasts were prepared as described¹⁴. Briefly, peripheral blood lymphocytes ($10^6\ \text{ml}^{-1}$) prepared as described (Fig. 1, legend) were activated for 72 h with UCHT1 antibody ($1\ \mu\text{g ml}^{-1}$). Thereafter cells (10^5 – $10^6\ \text{ml}^{-1}$) were maintained in the presence of exogenous recombinant Interleukin 2 (kindly provided by Professor K. A. Smith at $1\ \text{unit ml}^{-1}$ for 7–10 days before use.

lated polypeptides in untreated cells (Fig. 1, track 1). In contrast, a diffuse band of radioactivity of $M_r \sim 26\text{K}$ (Fig. 1, tracks 2–4) was maximally labelled in T3 immunoprecipitates after 5–15 min exposure to PHA with a decline in labelling at 40 min. It was coincident in position with the characteristically weakly ^{125}I -labelled T3 γ polypeptide (Fig. 1, track 5). A weakly phosphorylated polypeptide of $M_r \sim 20\text{K}$ was also observed that comigrated with the ^{125}I -labelled δ and ϵ subunits (Fig. 1, tracks 2–5). The 20K ^{32}P -labelled component was not reproducibly observed, but the 26K polypeptide was always present (Fig. 2*a*, track 2). Moreover, the 26K PHA-induced phosphorylated polypeptide was specific to UCHT1 immunoprecipitates, as it was never observed in immunoprecipitates of T11 and HLA-A,B,C antigens (Fig. 2*a*, tracks 3, 4). Contrastingly, a 20K phosphorylated component was frequently observed in control immunoprecipitations of T11 and HLA-A,B,C antigens (Fig. 2*a*, track 4) suggesting that it was not necessarily a specific component of the T3 complex.

To facilitate the designation of the ^{32}P -labelled polypeptides, T3 immunoprecipitates were digested with endoglycosidase F (endo-F). Under these circumstances the γ chain is reduced to partially and fully digested components of M_r 23K and 16K, respectively, whereas the δ chain yields a polypeptide of M_r 14K the ϵ chain is unaffected by endo-F^{6,7}. The results (Fig. 2*a*, tracks 5, 6) show that endo-F digestion of ^{32}P -labelled T3 isolated from PHA treated cells yielded a polypeptide of M_r 16K; no M_r 19K or 14K components were detected. It was concluded that PHA induces phosphorylation of the γ chain, but no concomitant reproducible phosphorylation of the δ or ϵ chains.

Previously, we have shown that phorbol-12, 13-dibutyrate (Pdbu) induces a strong phosphorylation of the T3 γ subunit and a weak phosphorylation of the δ chain (~ 7 -fold less)^{14,15}.

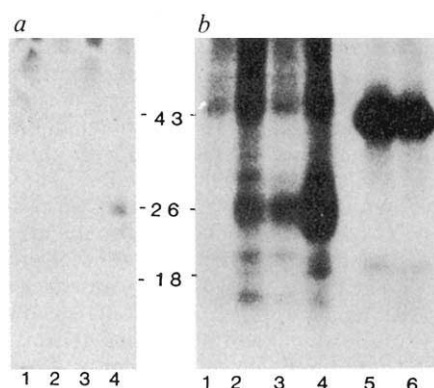


Fig. 3 *a*, Clone 19, a lymphocyte clone specific for influenza (Philippine) haemagglutinin (H3) was isolated and maintained in culture as previously described for other influenza specific T cell populations (ref. 24 and M.L., unpublished data). Cells were labelled with ^{32}P by incubation for 3 h at 37°C at $2 \times 10^6\ \text{ml}^{-1}$ in phosphate-free Eagle's medium supplemented with $80\ \mu\text{Ci ml}^{-1}$ ^{32}P -orthophosphate. Cells were then incubated for a further 15 min at 37°C alone (track 1); with $2 \times 10^6\ \text{ml}^{-1}$ EBVTL cells (donor homologous EBV-transformed cells) (track 2); with $1\ \mu\text{g ml}^{-1}$ of influenza (Chile) haemagglutinin (H1) plus $2 \times 10^6\ \text{ml}^{-1}$ EBVTL cells (track 3); or with $1\ \mu\text{g ml}^{-1}$ of influenza (Philippine) haemagglutinin (H3) plus $2 \times 10^6\ \text{ml}^{-1}$ EBVTL cells as antigen-presenting cells (track 4). Combinations of haemagglutinin plus EBVTL cells were preincubated together for 3 h at 37°C . Cells were lysed and T3 immunoprecipitates prepared and analysed as described (Fig. 1, legend). *b*, HA1.4, a T lymphocyte clone specific for synthetic peptides homologous to influenza haemagglutinin was isolated and maintained in culture as described²⁴. Cells were labelled with ^{32}P as described above and then incubated for a further 5 min alone (tracks 1, 5) or with $1\ \mu\text{g ml}^{-1}$ of peptide 14 (a 14 amino-acid peptide derived from the HA1 portion of influenza haemagglutinin), that had been preincubated with $2 \times 10^6\ \text{ml}^{-1}$ EBVTL cells as antigen-presenting cells, for 5 (tracks 2, 6) or 15 min (track 3), or with $50\ \text{ng ml}^{-1}$ of Pdbu for 15 min (track 4). T3 (tracks 1–4) and HLA-A, B, C immunoprecipitates (tracks 5, 6) were prepared and analysed as described in the legends to Figs 1 and 2.

In Fig. 2*b* (tracks 1, 2) a comparison of the extent of γ chain phosphorylation induced by PHA and Pdbu shows that Pdbu initiates a stronger T3 γ chain phosphorylation than PHA (~ 10 fold on the basis of densitometric scans). Thus, the failure to detect δ chain labelling in PHA treated cells could be due to the much lower level of T3 phosphorylation; any δ chain labelling could be below the limits of detection.

PHA could activate T cells via an interaction with Ti or the T11 antigen^{21–23}. Consequently, to explore whether recognition of antigen initiates T3 phosphorylation, we used cloned T cells (clone 19) reactive to influenza virus haemagglutinin (H3) that are restricted for class II DR4 molecules (M.L., unpublished data). When ^{32}P -labelled clone 19 cells were exposed to the haemagglutinin (H3) in the presence of antigen presenting cells, phosphorylation of the M_r 26K T3 γ polypeptide was observed (Fig. 3, track 4). No ^{32}P -labelled T3 γ was observed in untreated cells (Fig. 3*a*, track 1), nor when such cells were exposed to antigen-presenting cells alone (Fig. 3*a*, track 2) or antigen-presenting cells plus a non-stimulatory haemagglutinin (H1) (Fig. 3*a*, track 3). An identical phosphorylation of T3 γ was initiated by antigen stimulation for 5–15 min, of a cloned T cell population (HA1.4) that specifically recognises a 14-amino-acid peptide (p14) (Fig. 3*b*, tracks 2, 3) derived from the carboxy terminus of influenza virus haemagglutinin²⁴. The latter phosphorylation was not observed in T3 immunoprecipitates from unstimulated cells (Fig. 3*b*, track 1), or in immunoprecipitates of HLA-A,B,C antigens prepared from activated HA1.4 cells (Fig. 3*b*, tracks 5, 6). As noted previously with PHA-stimulated cells, Pdbu induced a stronger labelling than antigen of the 26K T3 γ polypeptide (Fig. 3*b*, track 4). Antigen and Pdbu also

induced phosphorylation of polypeptides of M_r 20K in UCHT1 immunoprecipitates, but these were not necessarily specific for T3 as similar ^{32}P -labelled polypeptides were observed in HLA-A,B,C immunoprecipitates (Fig. 3b, tracks 5, 6).

In conclusion, these data show that stimulation of human T cells with PHA or antigen is associated with phosphorylation of the γ subunit of the T3 antigen. A possible candidate for the immune regulated kinase is protein kinase C since activators of protein kinase C such as phorbol esters also initiate phosphorylation of the T3 antigen^{14,15}. The stimulation of protein kinase C moreover is anticipated as a normal consequence of T cell activation in that immune triggering of T cells is known to induce phosphatidylinositol breakdown and thus generate diacylglycerol, the proposed physiological activator of C kinase^{25,26}.

It has been shown previously that antigen and lectin stimulation of murine T cells are associated with phosphorylation of a M_r 20K–21K polypeptide that co-precipitates with the idiotype antigen receptor^{16,17}. A major question is whether, in spite of the apparent difference in M_r , this is analogous to the human T3 γ chain^{16,17,27}. Interestingly, one difference between the murine and human systems is that in murine T cells, phorbol esters induce phosphorylation of a M_r 25K component which, because of its lack of *N*-glycosylation, could be the murine T3 ϵ subunit¹⁶. In the human there is no evidence for phorbol ester-induced *in vivo* ^{32}P -labelling of the T3 ϵ chain¹⁵, although *in vitro* phosphorylation of the ϵ subunit can be readily demonstrated (unpublished data).

The functional relevance of intracellular signalling between protein kinase C and the T3 antigen is unclear. One possibility is that T3 γ chain phosphorylation is important in the primary activation pathways that initiate T cell growth and differentiation. It is noteworthy, however, that the immune activation of T cells by antigen, lectin, or phorbol ester is associated with a down-regulation of the surface levels of the Ti/T3 complex with a concomitant loss of antigen-regulated functions^{28–31}. Thus, one function of protein kinase C mediated T3 γ chain phosphorylation could be as a negative feedback signal that controls the level of expression and/or functions of the Ti/T3 complex.

We thank Gail Essery-Rice and Carol Greenall for technical assistance, Dr G. Janossy for providing the monoclonal antibody RFT11 and Kim Richardson for typing the manuscript.

Received 28 August; accepted 25 November 1986.

- Meuer, S. C. *et al.* *J. exp. Med.* **157**, 705–719 (1983).
- Acuto, O. *et al.* *Cell* **34**, 717 (1983).
- Meuer, S. C. *et al.* *Science* **222**, 1239–1241 (1983).
- Meuer, S. C. *et al.* *Nature* **303**, 808–810 (1983).
- Brenner, M. B., Trowbridge, J. S. & Strominger, J. L. *Cell* **40**, 183–190 (1985).
- Kanellopoulos, J. M., Wigglesworth, N. M., Owen, M. J. & Crumpton, M. J. *EMBO J.* **2**, 1807–1814 (1983).
- Borst, J., Alexander, S., Elder, J. & Terhorst, C. *J. biol. Chem.* **258**, 5135–5141 (1983).
- Van Waave, J. P., De Mey, J. R. & Goossens, J. G. *J. Immun.* **125**, 2708–2712 (1980).
- Chang, T. W., Kung, P. C., Gingsrus, S. P. & Goldstein, G. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1805–1808 (1981).
- Burns, G. R., Boyd, A. W. & Beverley, P. C. L. *J. Immun.* **129**, 1451–1457 (1982).
- Reinherz, E. L. *et al.* *Cell* **30**, 735–743 (1982).
- Oettgen, H. C., Terhost, C., Cantley, L. C. & Roscoff, P. M. *Cell* **40**, 583–590 (1985).
- Roscoff, P. M. & Cantley, L. W. *J. biol. Chem.* **260**, 14053–14059 (1985).
- Cantrell, D. A., Davies, A. A. & Crumpton, M. J. *Proc. natn. Acad. Sci. U.S.A.* **82**, 8158–8162 (1985).
- Davies, A. A., Cantrell, D. A. & Crumpton, M. J. *Biosciences* **5**, 867–876 (1985).
- Samelson, L. E., Harford, J. B., Schwartz, N. H. & Klausner, R. D. *Proc. natn. Acad. Sci. U.S.A.* **82**, 1969–1973 (1985).
- Samelson, L. E., Harford, J. B. & Klausner, R. D. *Cell* **43**, 223–231 (1985).
- Beverley, P. C. L. & Callard, R. E. *Eur. J. Immun.* **11**, 329–334 (1981).
- Walsh, F. S. & Crumpton, M. J. *Nature* **269**, 307–311 (1977).
- Barnstable, C. J. *et al.* *Cell* **14**, 9–20 (1978).
- Chilson, O. P., Boylston, A. W. & Crumpton, M. J. *EMBO J.* **3**, 3239–3245 (1984).
- O'Flynn, K., Krensky, A. M., Beverley, P. C. L., Burakoff, S. J. & Lynch, D. C. *Nature* **313**, 686–687 (1985).
- Ohashi, P. S. *et al.* *Nature* **316**, 606–609 (1985).
- Lamb, J. R., Eckels, D. D., Lack, P., Woody, J. U. & Green, N. *Nature* **300**, 66–69 (1982).
- Farrar, J. B. & Ruscetti, F. W. *J. Immun.* **136**, 1266–1273 (1985).
- Imboden, J. B. & Stobo, J. O. *J. exp. Med.* **161**, 446–456 (1985).
- Oettgen, H. C., Pettet, C. L., Maloy, L. & Terhorst, C. *Nature* **320**, 272–275 (1986).
- Reinherz, E. L. *et al.* *Cell* **30**, 735–743 (1982).
- Zanders, E., Lamb, J., Feldman, M., Green, N. & Beverley, P. *Nature* **313**, 318–320 (1983).
- Delia, D. *et al.* *Int. J. Cancer* **29**, 23–31 (1982).
- Ando, I., Harii, G., Wallace, D. & Beverley, P. *Euro. J. Immun.* **15**, 196–199 (1985).

Use of a cDNA clone to identify a supposed precursor protein containing valosin

Kerry J. Koller* & Michael J. Brownstein

Laboratory of Cell Biology, NIMH, Bethesda, Maryland 20892, USA

Valosin, a novel 25-amino-acid peptide isolated recently from pig intestine¹, has several effects on the digestive system of dogs². We report here that the valosin-specific complementary DNA clone from pigs codes for a polypeptide unlike most precursors of biologically active peptides. The predicted protein lacks a characteristic amino-terminal hydrophobic signal sequence and contains no processing signals of the type acted upon by endopeptidases to generate other active peptides from precursors. Antibodies to synthetic valosin have been used to show that nearly all valosin immunoreactivity is in the cytoplasm and that the protein detected (valosin-containing protein, VCP), although smaller than the predicted product of the cDNA sequence, is much larger than valosin. Valosin-specific messenger RNA is found in extracts from many pig tissues, which contrasts with the restricted occurrence expected of a biologically active peptide. We conclude that valosin is an artefact of the purification procedure and does not occur *in vivo*.

In recent years, Mutt and coworkers have isolated a number of biologically active peptides from pig gut extracts. These peptides have been identified by their biological activity or the presence of an amidated carboxy terminus³. The success of this approach is attested to by the large number of novel peptides that have been found, including peptide YY⁴, peptide HI⁵, galanin⁶, cholecystokinin-33⁷, secretin⁸, vasoactive intestinal polypeptide⁹ and motilin¹⁰. Many of the peptides discovered in the gut are also present in the brain illustrating the fact the transmitters used in the periphery are also often used in the central nervous system.

Recently, Schmidt *et al.* described the isolation, from porcine intestinal extracts, of valosin (Fig. 2b, residues 493–517), a novel 25-amino-acid peptide¹. It was purified from side fractions of an earlier preparation of peptide HI and secretin by its chromatographic and ultraviolet (UV)-absorbance characteristics and amino-terminal sequence. Its amino-acid sequence had no convincing sequence similarity with any known gut hormones (R. F. Doolittle, Databasem, San Diego), and it has several biological activities in dogs namely, release of gastrin; augmentation of pentagastrin-induced gastric secretion; stimulation of pancreatic protein secretion; and suppression of the

Valosin: 1 Val Gln Tyr Pro 5 Val Glu His Pro Asp Lys Phe Leu 10
Deduced
mRNA: 5' GTN-CAPu-TAPy-CCN-GTN-GAPu-CAPy-CCN-GAPy-AAPu-TTPy-PyTN 3'
Oligonucleotide
probe: 3' CAT/G-GTC-ATG-GGT/G-CAT/G-CTT-GTT-GGT/G-CTG-TTT-AAG-GA 5'
cDNA: 5' GTC-CAG-TAT-CTT-GTG-GAG-CAC-CCA-GAC-AAA-TTC-CTC 3'

Fig. 1 Oligonucleotide probe for valosin. The first 12 amino acids in valosin were used to design the 35-residue oligonucleotide probe. All possible mRNAs encoding this portion of valosin are represented in the second line of the figure. N is A, C, G and T; Pu is A and G; Py is T and C. The 16-fold degenerate oligonucleotide (line 3) was synthesized on an Applied Biosystems 380A DNA synthesizer according to the manufacturer's instructions and purified on a preparative denaturing acrylamide gel. The resulting mixture of probes was labelled with [γ - ^{32}P]ATP using T4 DNA kinase²¹. Line four shows the segment of the sequence of the isolated valosin cDNA complementary to the probe. Nucleotides that differed from the probe, which is 85.7% homologous to the cDNA, are underlined.

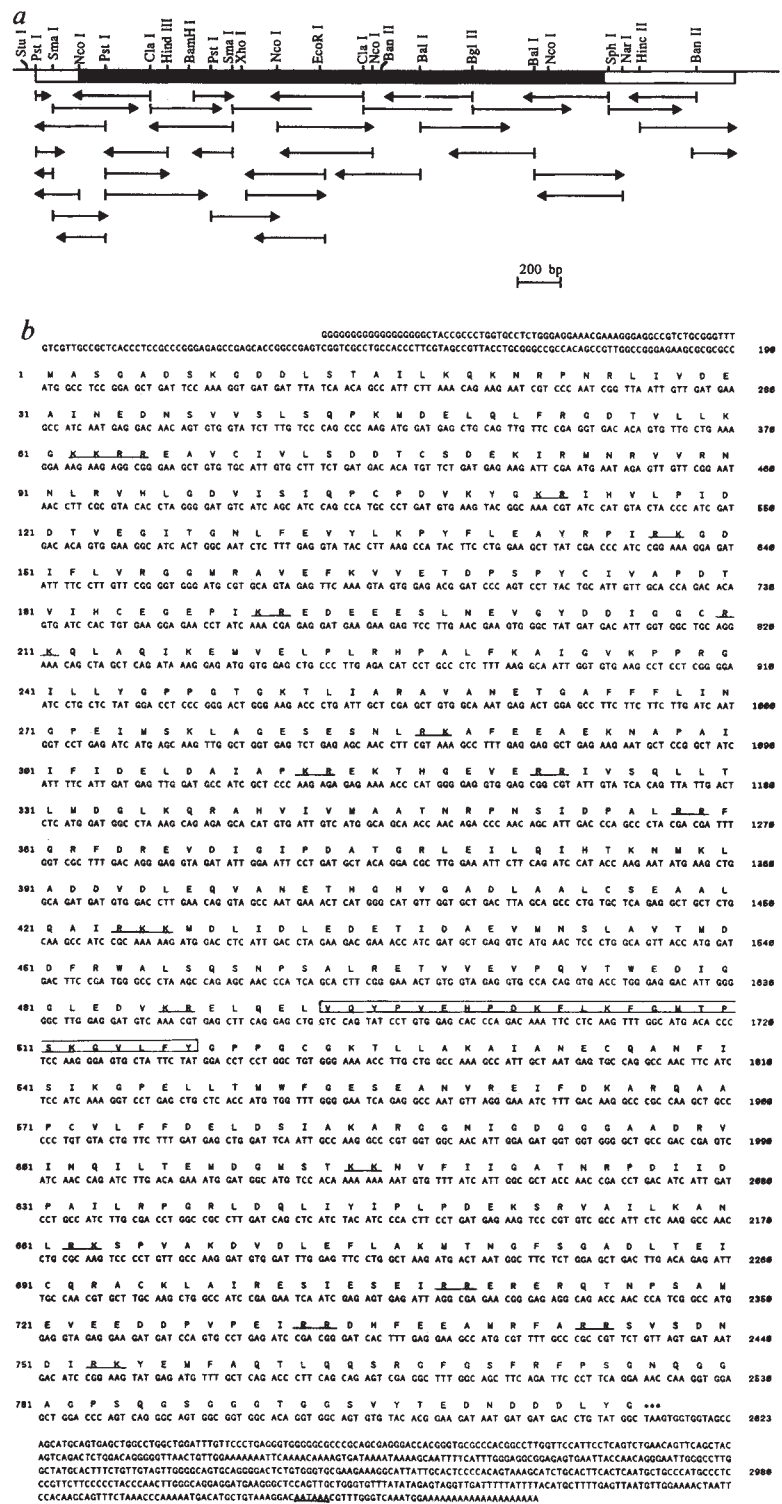


Fig. 2 Determination of the primary sequence of the valosin precursor mRNA. *a*, Restriction map and sequencing strategy used to characterize the largest valosin precursor clone, pcD-VQY 3-1. Horizontal arrows indicate the direction of sequencing and the location and length of sequenced regions. DNA fragments were subcloned into M13mp18 or 19, and the isolated single-stranded templates were used for sequencing by the dideoxy chain-termination method²². Open bar, total cDNA insert; dark bar, open reading frame. *b*, Nucleotide sequence of the cDNA insert from clone pcD-VQY 3-1 and the predicted amino-acid sequence of the valosin precursor. The sequence of the isolated valosin peptide is boxed. Possible paired basic amino-acid processing signals are underlined, as is the polyadenylation signal. Amino acids are numbered on the left and nucleotides on the right.

migrating myoelectric complexes of the small bowel in the fasting state².

Here we describe the characterization of the mRNA encoding valosin. An Okayama-Berg porcine adrenal medulla complementary DNA library¹¹ was screened with an oligonucleotide probe (Fig. 1) by filter hybridization¹². Six positive clones were identified and purified by sequential low-density plating. The largest cDNA (pcD-VQY 3-1, 3.2-kilobase (kb) insert size) was studied in detail. The restriction map and sequencing strategy used to characterize pcD-VQY 3-1 are shown in Fig. 2a, and the nucleotide and corresponding deduced amino-acid sequences in Fig. 2b. The cDNA contains a 2,466 base pair (bp)

open reading frame flanked by 143 and 550 bp of 5' and 3' untranslated sequences, respectively. The nucleotide and the predicted amino-acid sequences were not homologous to known sequences in either the Genbank or the NBRF Georgetown Protein databases.

Every tissue examined by Northern blot analysis had a single RNA message for valosin of ~3.4 kb (Fig. 3). Thus, clone pcD-VQY 3-1 is nearly full length. Message was found not only in porcine adrenal medulla, ileum and frontal cerebral cortex, but also in cerebellum, caudate, spinal cord, adrenal cortex, heart and liver. Valosin message was also present in many neuronal cells of porcine spinal cord, cerebellum and hippocam-

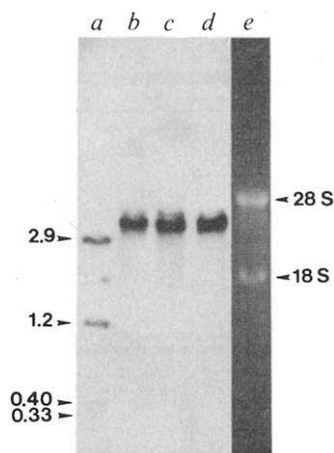


Fig. 3 Northern blot analysis of the tissue distribution of valosin mRNA. Lanes: a, θ X 174 RF DNA digested with *Taq*I and end-labelled with 32 P-dCTP and large fragment DNA polymerase²¹, denatured in formamide at 56 °C for 15 min before loading. Sizes in kb, b, ileum extract; c, adrenal medulla extract; d, frontal cerebral cortex extract; e, ethidium bromide staining of total ileum extract; RNA.

Methods. Porcine tissue (from the NIH animal facility, Poolesville, Maryland) was frozen on dry ice and stored at -70 °C until used for RNA extraction in guanidium thiocyanate²³. Total RNA (5 μ g) was suspended in denaturing buffer and electrophoresed on a 1% agarose formaldehyde denaturing gel and the RNA transferred to Gene Screen (NEN). The *Eco*RI-*Sph*I (1.3 kb) fragment (see Fig. 2a) was 32 P-labelled using the Feinberg random primer method²⁴ to a specific activity of 9.3×10^8 d.p.m. per μ g. The filter was pre-hybridized for 8 h at 45 °C in $5 \times$ SSPE, $1 \times$ Denhardt's, 50% formamide, 0.1% SDS, 250 μ g ml⁻¹ tRNA, 124 μ g ml⁻¹ single-stranded, denatured salmon sperm DNA. After addition of denatured, labelled probe, hybridization was continued for 40 h in the same conditions. The filter was washed in $0.1 \times$ SSPE, 0.1% SDS at 65 °C and placed against X-ray film in a cassette equipped with two intensifying screens for 36 h at -70 °C.

pus, and in most cells of the adrenal gland, liver, and intestinal mucosa as determined by *in situ* hybridization histochemistry¹³. This almost ubiquitous distribution of valosin mRNA is unusual; a message that encodes a biological active peptide would be expected to be present in selected neurons and neuroendocrine cells.

The structure of the predicted valosin-containing protein (VCP) (Fig. 2b) is not typical for a peptide precursor. These proteins characteristically have a hydrophobic amino-terminal signal sequence required for their entry into the cisternal space of the rough endoplasmic reticulum from where the propeptide travels to the Golgi apparatus. In addition, within their precursors, the biologically active peptide or peptides are flanked by processing signals such as paired basic amino acids, at which a trypsin-like endopeptidase acts to liberate them. The cDNA sequence of VCP encodes a molecule of 806 amino acids (relative molecular mass (M_r) ~88,660 (Fig. 2b). The complete amino-acid sequence for valosin itself is encoded by nucleotides 1,667 to 1,741 (amino acids 493 to 517 of VCP). Valosin is not bracketed by canonical processing signals. Furthermore, although the amino terminus of VCP contains a few possible signal peptide cleavage sites¹⁴, it is not very hydrophobic. Analysis of the complete amino-acid sequence of VCP using the algorithm of Hopp and Wood¹⁵ revealed no putative internal signal sequences either. The above analyses suggest that VCP or its products are not secreted and that VCP is likely to be a cytoplasmic protein. Valosin-specific antiserum produced by injecting rabbits with synthetic valosin conjugated to bovine serum albumin (BSA) and purified on protein A- and BSA-affinity columns was used to immunoprecipitate subcellular fractions of porcine adrenal gland, and showed that >95% of

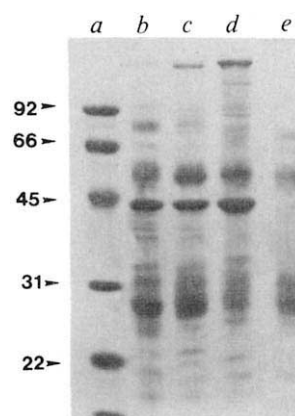


Fig. 4 Immunoprecipitation of valosin-containing proteins from porcine tissue. Lanes: a, Biorad protein size standard, M_r as shown; b, ileum; c, adrenal medulla; d, frontal cerebral cortex; e, control.

Methods. Frozen porcine ileum, adrenal medulla and frontal cerebral cortex were ground into powder under liquid N₂ and homogenized in RIPA buffer (150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 10 mM Tris-HCl, pH 7.2) with 1% trasyol as protease inhibitor in a glass-glass homogenizer. For controls, RIPA buffer (1 μ l) plus 1% trasyol without additional tissue was carried through the following procedures. Samples were spun at 100,000 g to clarify. Supernatants were removed and precleared with 200 μ l of Staph A cell-suspension. The samples were incubated with 100 μ l of valosin-specific protein A- and BSA-affinity purified serum (206 AP-686) on ice for 2.5 h. Staph A cells (200 μ l) were added to the samples and incubated overnight at 4 °C. Samples were spun at 4 °C in a microcentrifuge for 10 min and pellets washed three times with RIPA buffer (1 ml) plus trasyol. The final pellet was suspended into 40 μ l Laemmli sample buffer²⁵ with 5% β -mercaptoethanol and placed on ice for 1 h. Samples were boiled for 3 min, placed on ice for 5 min, and spun in microcentrifuge for 10 min at 4 °C. Supernatant was removed and samples (equivalent to 25 mg of soluble protein before immunoprecipitation) were run on a 10%, 1.5-mm polyacrylamide protein gel²⁵. Gel was stained for 10 min in 0.25% Coomassie blue in 50% methanol, 10% acetic acid, and destained for 3 h in 20% methanol, 7% acetic acid.

the immunoreactivity was present in the cell cytoplasm (data not shown).

Although VCP does not appear to be a peptide precursor, it does seem to undergo post-translational modification. The mRNA predicts a protein product of M_r ~88,660. The *Stu*I-*Sph*I fragment of the cDNA encoding the complete open reading frame was subcloned into the GEM-3 riboprobe vector (Promega Biotec) and after *in vitro* transcription¹⁶, the synthesized RNA was translated *in vitro* with nuclease-treated rabbit reticulocyte lysate (NEN)¹⁷. A number of proteins of different sizes were immunoprecipitated, presumably resulting from initiation of translation at various methionine residues. The largest protein band was of M_r ~90,000, the probable size of the initial protein translated *in vivo*, as the sequence flanking the proposed AUG initiating codon (GCCAUGG) is an optimal sequence for initiation of translation by eukaryotic ribosomes¹⁸. However, the protein species immunoprecipitated from tissue extracts of porcine adrenal medulla, ileum and frontal cerebral cortex was only of M_r 43,000 (Fig. 4). These results suggest that the VCP of M_r 90,000 is processed *in vivo* into a species of M_r 43,000 that is recognized by the valosin-specific antibody. We believe that valosin is a product of chymotryptic degradation of VCP and may have arisen in the course of extraction or purification.

Thus, our findings suggest that the recently described porcine gut peptide, valosin, is an artefactual product of a hitherto unknown cytoplasmic protein generated from a larger protein (VCP). Although preliminary experiments indicated that valosin may be biologically active, other protein degradation products

have also been shown to have biological activity (for example, fragments of the α chain of haemoglobin)^{19,20}, and care must be exercised before predicting that a novel peptide has a physiological role. Studies of cDNA of putative peptide 'precursors' may help identify strong candidates and eliminate weak ones.

We thank Anna Iacangelo, Alice Young and Drs Tom Bonner, Urs Affolter and Åke Rökaeus for technical insights; Dr Åke Rökaeus for providing the cDNA library; Dr Scott Young for his help with *in situ* hybridization histochemistry; Dr Wolfgang Schmidt for providing the complete structure of valosin before publication; Dr Dan Marshak and Joanne Gutierrez for providing the synthetic peptide; and Drs Dianne Rausch and Maribeth Eiden for critical review of the manuscript. K.J.K. was supported by a National Institute of Neurological and Communicative Diseases and Stroke (NINCDS) NRSA postdoctoral grant no. NS07599.

Received 5 September; accepted 1 December 1986.

- Schmidt, W. E. *et al.* *FEBS Lett.* **191**, 264–268 (1985).
- Schmidt, W. E. *et al.* *Dig. Dis. Sci.* **29**, 75S (1984).
- Tatemoto, K. & Mutt, V. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4115–4119 (1978).
- Tatemoto, K. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2514–2518 (1982).
- Tatemoto, K. & Mutt, V. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6603–6607 (1981).
- Tatemoto, K., Rökaeus, Å., Jörnvall, H., McDonald, T. J. & Mutt, V. *FEBS Lett.* **164**, 124–128 (1983).
- Mutt, V. in *Gastrointestinal Hormones* (ed. Glass, G. B. J.) 169–180 (Raven, New York, 1980).
- Jorpes, J. E., Mutt, V., Magnusson, S. & Steele, B. B. *Biochem. biophys. Res. Commun.* **9**, 275–278 (1962).
- Said, S. I. & Mutt, V. *Eur. J. Biochem.* **28**, 199–204 (1972).
- Brown, J. C., Cook, M. A. & Oryburgh, J. R. *Gastroenterology* **62**, 401–404 (1972).
- Rökaeus, Å. & Brownstein, M. J. *Proc. natn. Acad. Sci. U.S.A.* **83**, 6287–6291 (1986).
- Grunstein, M. & Hogness, D. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3961–3965 (1975).
- Young, W. S. III, Mezey, E. & Siegel, R. E. *Neurosci. Lett.* **70**, 198–203 (1986).
- Von Heijne, G. *Nucleic Acids Res.* **14**, 4683–4690 (1986).
- Hopp, T. P. & Woods, K. R. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3824–3828 (1981).
- Melton, D. A. *et al.* *Nucleic Acids Res.* **12**, 7035–7056 (1984).
- Pelham, H. R. B. & Jackson, R. J. *Eur. J. Biochem.* **67**, 247–256 (1976).
- Kozak, M. *Cell* **44**, 283–292 (1986).
- Schally, A. V. *et al.* *Biochem. biophys. Res. Commun.* **82**, 582–588 (1978).
- Kiso, Y. *et al.* *FEBS Lett.* **155**, 281–284 (1983).
- Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning; A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
- Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).
- Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18**, 5294–5299 (1979).
- Feinberg, A. P. & Vogelstein, B. *Analyt. Biochem.* **137**, 266–267 (1984).
- O'Farrell, P. J. *biol. Chem.* **250**, 4007–4021 (1975).

Cloning of decay-accelerating factor suggests novel use of splicing to generate two proteins

Ingrid W. Caras, Michael A. Davitz*, Lucy Rhee, Greg Weddell, David W. Martin, Jr & Victor Nussenzweig*

Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA

*Departments of Pathology and †Environmental Medicine, New York University School of Medicine, New York, New York 10016, USA

Decay-accelerating factor (DAF), a glycoprotein that is anchored to the cell membrane by phosphatidylinositol^{1,2}, binds activated complement fragments C3b and C4b, thereby inhibiting amplification of the complement cascade on host cell membranes^{3–5}. Here, we report the molecular cloning of human DAF from HeLa cells. Analysis of DAF complementary DNAs revealed two classes of DAF messenger RNA, one apparently derived from the other by a splicing event that causes a coding frameshift near the C terminus. The apparent 'intron' sequence contains an *Alu* family member and encodes contiguous protein sequence. Two DAF proteins are therefore possible, having divergent C-terminal domains which differ in their hydrophobicity. Both mRNAs are found on polysomes, suggesting that both are translated. We

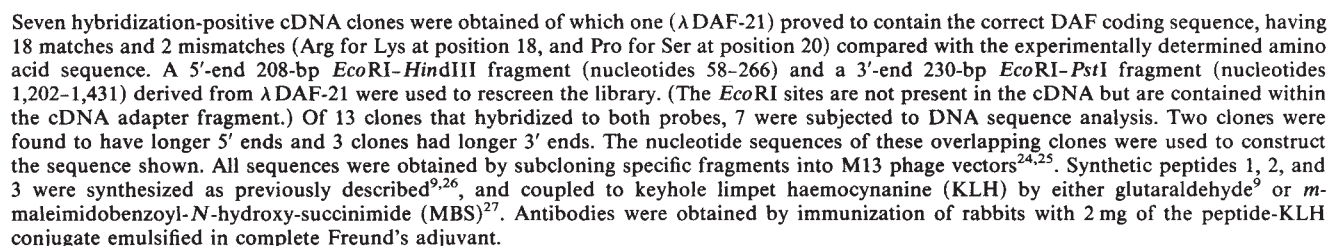
propose that the major (90%) spliced DAF mRNA encodes membrane-bound DAF whereas the minor (10%) unspliced DAF mRNA may encode secreted DAF and we present expression data supporting this. The deduced DAF sequence contains four repeating units homologous to a consensus repeat found in a recently described family of complement proteins⁶.

A 69-base synthetic oligonucleotide 'long-probe'^{7,8} based on the N-terminal sequence of DAF⁹ was used to screen a λ gt10 cDNA library (1×10^6 recombinants) derived from HeLa cell poly (A)⁺ RNA. Of several hybridizing clones, a single 1,395-base-pair (bp) clone was shown by DNA sequence analysis to encode the known amino-acid sequence. Northern analysis of DAF mRNA revealed two major mRNA bands of ~1.5 and ~2.2 kilobases (kb). We therefore rescreened the library and identified several longer clones. The complete sequence of the cDNA and deduced protein are shown in Fig. 1a. To confirm that this cDNA encodes DAF, we raised anti-peptide antibodies against the deduced peptides 1, 2, and 3 (Fig. 1a), and showed by immunoblotting that all three antibodies specifically recognize the relative molecular mass 70,000 (M_r 70 K) DAF protein purified from human erythrocytes (Fig. 1b).

Two classes of DAF cDNA clones were identified by DNA sequence analysis. These differed by an apparent internal deletion of 118 bp which probably represents a splicing event (see below). The translation initiation codon was assigned to the first in-frame ATG which is flanked by sequences that conform to Kozak's rules¹⁰. This predicts a hydrophobic signal peptide of 34 amino acids preceding the known N terminus of DAF. The deduced mature DAF contains four contiguous repeating units of ~63 amino acids (Fig. 1c) that conform to a consensus repeat sequence found in the complement proteins Factor B, C2, Factor H, C4b-binding protein, CR1, and C1r (all of which bind C3 or C4), and in a few non-complement proteins including the IL-2 (interleukin-2) receptor and a subunit of clotting factor XIII⁶. Outside the general consensus framework, the first and second repeats of DAF show significant homology (~38%), as do the third and fourth (~40%), but the homology of repeats 1 or 2 compared with 3 or 4 is weaker. This suggests that these repeats arose by sequential internal duplications. The deduced protein contains one site for N-linked glycosylation (Asn, residue 61). Several clusters of threonine and serine residues flank the repeats and may be sites of O-linked glycosylation^{11,12}.

Three out of seven clones sequenced contained a precise 118-bp deletion within the C-terminal portion of the coding region. The sequences bordering this deletion match the 5' and 3' consensus sequences for RNA splicing^{13,14}, suggesting that the deleted segment represents an unspliced intron. Interestingly, an *Alu* family sequence displaying an 80% homology with the *Alu* consensus¹⁵ lies within this apparent intron (Fig. 1a). The intron contains no stop codons so that the unspliced DAF mRNA could encode a protein of 440 amino acids (including signal peptide) of which 39 residues (328–366) near the C terminus would be encoded by the intron. Removal of the intron by splicing causes a frameshift in the C-terminal coding region, resulting in a shorter protein of 381 amino acids with a different C terminus (Figs 1a and 2a). The hydropathy profiles of the two DAF proteins encoded by the spliced or unspliced DAF mRNAs differ markedly in the divergent C-terminal domains, being hydrophobic in the case of DAF deduced from the spliced mRNA (short protein), or hydrophilic in DAF from the unspliced mRNA (long protein) (Fig. 2b, c). This difference may have implications for the cellular localization of DAF.

Each class of DAF cDNA displays heterogeneity at the 3' end. Four polyadenylation signals, AATAAA¹⁶, are present in the 3' noncoding region of the sequence. DNA sequence analysis of several independent clones revealed that at least the first and last of these sites are used, producing 3' noncoding regions differing in size by 788 bp, resulting in the two major mRNA species (~2.2 and ~1.5 kb) identified by Northern analysis (Fig. 3a, lane 1). To confirm this, we rescreened the Northern blot



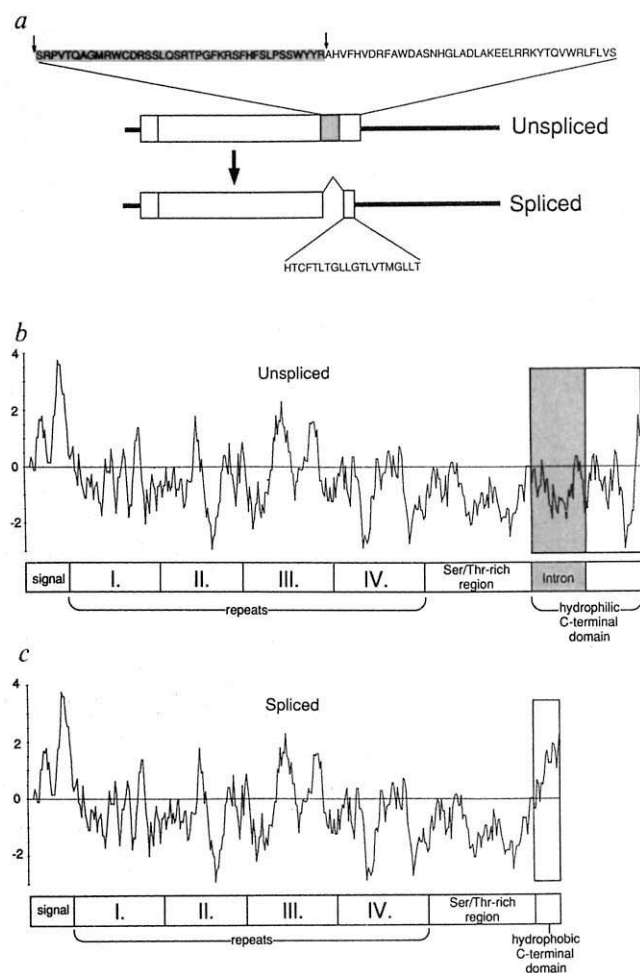


Fig. 2 Schematic representation of DAF mRNAs and deduced protein domains. *a*, Schematic representation of unspliced and spliced DAF mRNAs with coding sequences boxed. The shaded area denotes the 118-bp intron that is spliced out. The divergent C-terminal amino-acid sequences deduced from each mRNA are shown. The shaded amino acids indicate intron-encoded protein sequence. *b*, *c*, Hydropathy analysis and schematic representation of deduced protein domains. The deduced amino-acid sequences derived from the unspliced (*b*) or spliced (*c*) mRNAs were scanned using the computer program of Kyte and Doolittle²⁸. Landmarks in the sequence are indicated schematically. The divergent C-terminal domains of the long (unspliced mRNA) and short (spliced mRNA) proteins are boxed and the intron (*Alu* sequence)-encoded area is shaded. I-IV denote the homologous repeating units shown in Fig. 2*b*.

with a restriction fragment located between the first and last polyadenylation sites. As predicted, this probe hybridized only to the ~2.2 kb mRNA (Fig. 3*a*, lane 2).

To determine whether the unspliced DAF cDNA was derived from a nuclear precursor or from cytoplasmic mRNA, 22-mer oligonucleotide probes designed to distinguish spliced from unspliced DAF mRNA were used to probe cytoplasmic poly(A)⁺ RNA from HeLa cells (Fig. 3*b*). Both forms of DAF mRNA were readily detectable. The spliced mRNA is more abundant (90% of DAF mRNA), consisting mainly of two species of ~1.5 and ~2.2 kb (lane 4). The less abundant (10%) unspliced mRNA favours the first polyadenylation site, producing predominantly a ~1.6 kb (that is 1.5 + 0.118) species (lane 3), although sequence analysis of several cDNA clones suggests that the downstream site is also used. To determine whether both classes of mRNA are translated, we analysed poly(A)⁺ RNA extracted from HeLa cell polysomes (Fig. 3*c*). We detected both the spliced and

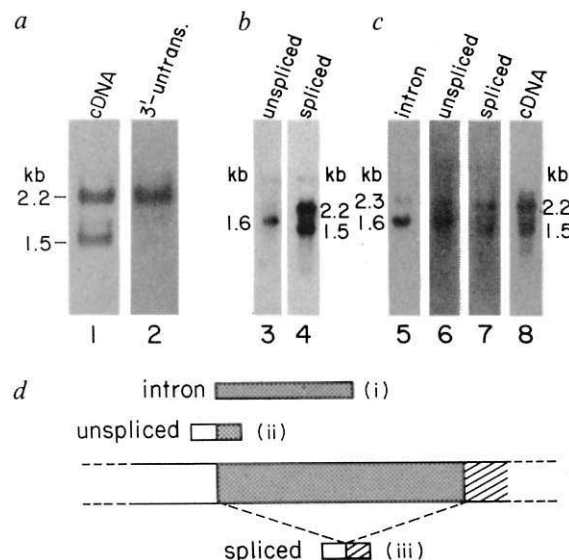


Fig. 3 Northern analysis of HeLa cell RNA. *a*, *b*, Northern analysis of cytoplasmic HeLa cell poly(A)⁺ RNA. Lane 1 was hybridized with a 1,395-bp DAF cDNA probe (the insert from λ DAF-21), and lane 2 with a 246 bp 3' *HincII-EcoRV* fragment (nucleotides 1,473-1,719) located between the first and last polyadenylation sites in the 3'-untranslated region; lanes 3 and 4 were hybridized with 22-mer probes (ii) and (iii) (see Fig. 3*d*) specific for unspliced or spliced DAF mRNA respectively. *c*, Northern analysis of poly(A)⁺ RNA from HeLa cell polysomes. Lane 5 was hybridized with an intron-specific 64-mer (probe (i)), lanes 6 and 7 with 22-mer probes (ii) and (iii) specific for unspliced or spliced DAF mRNA respectively, and lane 8 with a full-length DAF cDNA probe. *d*, Schematic representation of the synthetic oligonucleotide probes designed to detect either unspliced or spliced DAF mRNA specifically. Probe (i), 64-mer (nucleotides 1,147-1,210) specific for the intron region of the sequence; probe (ii), 22-mer spanning the first exon/intron boundary (3'-AGAAGATAGACCAAGAG-CAGGA-5', complementary to nucleotides 1,136-1,157), specific for unspliced DAF mRNA; probe (iii), 22-mer spanning the splice junction (3'-AGAAGATAGACCCGTGTGCACA-5', complementary to nucleotides 1,136-1,146 and 1,265-1,275), specific for spliced DAF mRNA.

Methods. Polyadenylated RNA (1-2 μ g) was electrophoresed into a formaldehyde-1% agarose gel²⁹ and blotted onto Gene Screen membrane according to manufacturer's instructions. The preparation of RNA from polysomes was as described³⁰. The ³²P-labelling of probes was carried out using T4 polynucleotide kinase for synthetic oligonucleotides³¹ and DNA polymerase for cDNA fragments³². The 64-mer and cDNA probes were hybridized in 50% formamide, 10% dextran sulphate, 5 $\times$ SSC, 10 $\times$ Denhardt's, 20 mM sodium phosphate pH 7.0, 100 μ g ml⁻¹ salmon sperm DNA, 0.1% SDS at 42 $^{\circ}$ C. Washes were performed at 55-60 $^{\circ}$ C in 0.2 $\times$ SSC, 0.2% SDS. The 22-mer probes were hybridized in the above hybridization solution without formamide, at 37 $^{\circ}$ C. Washes were performed in 3 M tetramethylammonium chloride solution at 56 $^{\circ}$ C (ref. 33). These conditions permit hybridization only with a perfectly matched probe (verified experimentally).

unspliced mRNAs using as probes both the two 22-mers and a 64-mer specific for the intron sequence. This result suggests that both DAF mRNAs are translated in HeLa cells, giving rise to two DAF proteins that diverge at their C termini. We have also detected the unspliced DAF mRNA in a prostate carcinoma (data not shown), so the existence of spliced and unspliced DAF mRNAs is not specific to HeLa cells.

The system apparently used to generate these divergent proteins has a number of novel features. First, a frameshift caused by a splicing event produces a switch from a hydrophilic to hydrophobic sequence. Second, the 'intron' has potential coding function. Third, this appears to be the first case in which an *Alu* sequence (contained within the intron) encodes protein.

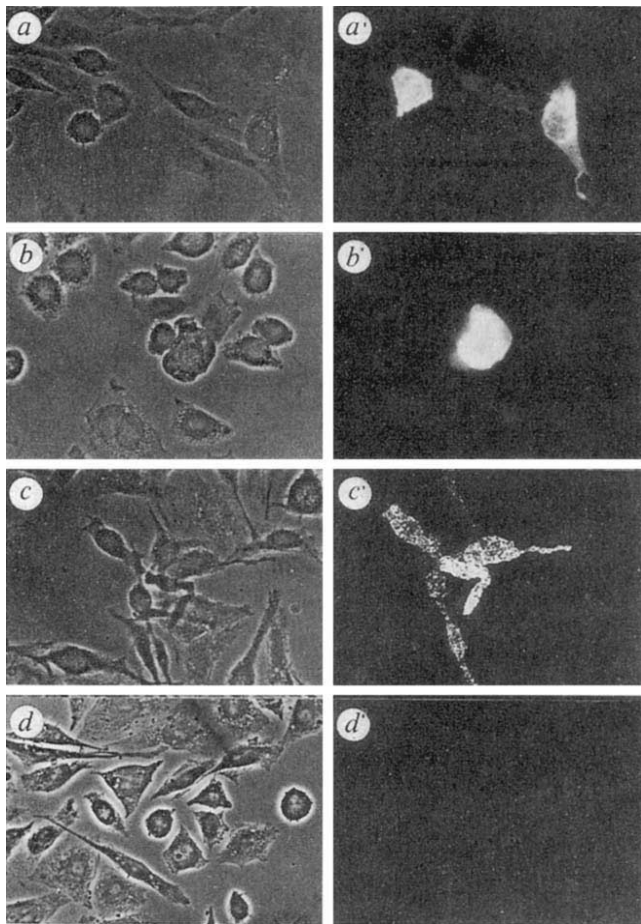


Fig. 4. Detection of human DAF by immunofluorescent labelling of transfected Chinese hamster ovary (CHO) cells. CHO cells were transfected with the plasmid pRSV.DAF, containing the spliced DAF cDNA (a and c), or with pRSV.DAF.I, containing the unspliced DAF cDNA (b and d). Panels a and b show lysed cells labelled internally. Panels c and d show cell surface labelling of intact cells. Paired phase (a, b, c, d) and rhodamine fluorescence (a', b', c', d') photographs were made with Ilford XPI film. No labelled cells were seen in non-transfected CHO controls (data not shown).

Methods. Full-length DAF cDNAs (spliced or unspliced) were assembled from overlapping clones and inserted into a mammalian expression vector between an RSV promoter and an SV40 polyadenylation sequence³⁴. In addition, the plasmids contained a mouse dihydrofolate reductase (DHFR) cDNA transcribed by an SV40 early promoter to provide a selectable marker for gene expression³⁵. The plasmids were transfected into DHFR⁻ CHO cells by the calcium-phosphate coprecipitation method^{35,36} and selected clones were pooled. For internal labelling, cells were fixed for 5 min in 3.7% formaldehyde and then permeabilized with ethanol or 0.1% Triton X-100/phosphate-buffered saline for 5 min^{37,38}. For surface labelling, cells were either fixed in formaldehyde or incubated directly with antibody^{37,38}. Cells were incubated with monoclonal antibodies against DAF (specific for human DAF⁵) for 2–4 h at 37 °C, washed in phosphate-buffered saline and exposed to rhodamine-conjugated goat anti-mouse IgG for 2 h.

DAF occurs in nature largely as a membrane-bound glycoprotein, anchored to the cell surface membrane by a glycosphospholipid (phosphatidylinositol)^{1,2}. A soluble form of DAF of unknown function or origin has also been detected in many body fluids¹⁷. Of the phospholipid-anchored membrane proteins reviewed in ref. 18, Thy-1^{19,20} and the variant surface glycoproteins (VSGs)²¹ of *Trypanosoma brucei* are initially synthesized with a hydrophobic C-terminal peptide that is cleaved and replaced by a phosphatidylinositol-containing gly-

cophospholipid anchor. We therefore speculated that spliced DAF mRNA, which predicts a protein with a hydrophobic C terminus, encodes the membrane form of DAF (presumably the hydrophobic peptide is cleaved and replaced with a glycosphospholipid). Unspliced DAF mRNA might then encode a soluble form of DAF which, lacking a hydrophobic C-terminal peptide, could be secreted directly into the fluid phase. Interestingly, the spliced mRNA encodes a cysteine immediately preceding the hydrophobic C-terminal domain. The glycosphospholipid moiety of Thy-1 is known to attach to a cysteine located upstream of the cleaved hydrophobic C-terminal domain²⁰.

To test the above hypothesis we constructed mammalian expression vectors containing either the spliced or the unspliced DAF cDNA under control of the Rous sarcoma virus (RSV) promoter, and transfected these plasmids into Chinese hamster ovary (CHO) cells. Stably transfected clones were pooled and analysed by immunofluorescent labelling using mouse monoclonal antibodies specific for human DAF. Both the spliced and the unspliced DAF cDNAs directed the expression of human DAF in CHO cells as shown by immunofluorescent labelling of fixed, permeabilized cells (Fig. 4a, b). In contrast, surface labelling of non-permeabilized cells (fixed or untreated) indicated that cells transfected with the spliced DAF cDNA expressed human DAF on the cell surface whereas cells containing the unspliced cDNA did not (Fig. 4c, d). These results are consistent with the hypothesis that membrane-bound and soluble DAF are encoded by the spliced and unspliced DAF mRNAs, respectively. However, we have not yet been able to detect the putative secreted species *in vivo*.

Differential RNA splicing as a means of affecting cellular localization has been described for IgM molecules²². Cells switch from making membrane-bound to secreted antibodies by selection of either a hydrophobic or hydrophilic C-terminal segment. The DAF case is mechanistically different. The divergent C-terminal coding regions are overlapping and in different reading frames; those of the IgM heavy chain are distinct.

We thank Mark Vasser, Peter Ng, and Parkash Jhurani for oligonucleotide synthesis, Lisa Coussens for the cDNA library, John Burnier and David Schlesinger for synthetic peptide synthesis, Carol Morita and Alane Gray for illustrations, and David Standing and John McLean for discussions. This work was supported by Genentech Inc., by NIH grant AI-08499 to V.N. and by a Physician-Scientist Award (NIEHS1K12ES001136-01) to M.D.

Received 30 October; accepted 24 November 1986.

1. Davitz, M. A., Low, M. G. & Nussenzweig, V. *J. exp. Med.* **163**, 1150–1161 (1986).
2. Medof, M. E., Haas, R., Walter, E. I. & Rosenberry, T. L. *Fed. Proc.* **45**, 382 (1986).
3. Nicholson-Weller, A. J. *et al. J. Immun.* **129**, 184–189 (1982).
4. Medof, M. E., Kinoshita, T. & Nussenzweig, V. *J. exp. Med.* **160**, 1558–1578 (1984).
5. Kinoshita, T., Medof, M. E. & Nussenzweig, V. *J. Immun.* **136**, 3390–3395 (1986).
6. Reid, K. B. M. *et al. Immun. Today* **7**, 230 (1986).
7. Anderson, S. & Kingston, I. B. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6836–6842 (1983).
8. Ullrich, A. *et al. Nature* **316**, 418–425 (1984).
9. Davitz, M. A., Schlesinger, D. & Nussenzweig, V. *J. Immun. Meth.* (in the press).
10. Kozak, M. *Microbiol. Rev.* **57**, 1–45 (1983).
11. Cummings, R. D. *et al. J. biol. Chem.* **258**, 15261–15273 (1983).
12. Russell, D. W. *et al. Cell* **37**, 577–585 (1984).
13. Mount, S. M. *Nucleic Acids Res.* **10**, 459–472 (1982).
14. Sharp, P. A. *Cell* **23**, 643–646 (1981).
15. Schmidt, C. W. & Jelinek, W. R. *Science* **216**, 1065–1070 (1982).
16. Proudfoot, N. J. & Brownlee, G. G. *Nature* **263**, 211–214 (1976).
17. Medof, M. E. *et al. Complement* **2**, 53 (1985).
18. Low, M. G., Ferguson, M. A. J., Futerman, A. H. & Silman, I. *Trends biochem. Sci.* **11**, 212–215 (1986).
19. Low, M. G. & Kincade, P. W. *Nature* **318**, 62–64 (1985).
20. Tse, A. G. D. *et al. Science* **230**, 1003–1008 (1985).
21. Ferguson, M. A. J. *et al. J. biol. Chem.* **261**, 356–362 (1986).
22. Early, P. *et al. Cell* **20**, 313–319 (1980).
23. Towbin, H., Staehlin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350 (1979).
24. Messing, J., Crea, R. & Seeberg, P. H. *Nucleic Acids Res.* **9**, 309–321 (1981).
25. Sanger, F., Nickle, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).
26. Vergara, U. *et al. Molec. biochem. Parasitol.* **14**, 283–292 (1985).
27. Green, N. *et al. Cell* **28**, 477–487 (1982).
28. Kyte, J. & Doolittle, R. F. *J. molec. Biol.* **157**, 105 (1982).
29. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning; a Laboratory Manual*, 202–203 (Cold Spring Harbor Laboratory, New York, 1982).
30. Goddard, J. M., Caput, D., Williams, S. R. & Martin, D. W., Jr. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4281–4285 (1983).

31. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: a Laboratory Manual*, 122-124 (Cold Spring Harbor Laboratory, New York, 1982).
32. Taylor, J. M., Illmensee, R. & Summers, S. *Biochim. biophys. Acta* **442**, 324-330 (1976).
33. Wood, W. I. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 1585-1588 (1985).
34. Gorman, C. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 6777-6781 (1982).
35. Simonsen, C. C. & Levinson, A. D. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2495-2499 (1983).
36. Wigler, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 1373-1376 (1979).
37. Gottlieb *et al. J. Cell Biol.* **102**, 1242-1255 (1986).
38. Rose, J. K. & Bergmann, J. E. *Cell* **30**, 753-762 (1982).

Transforming potential of the *c-fms* proto-oncogene (CSF-1 receptor)

Martine F. Roussel,* Thomas J. Dull,†
Carl W. Rettenmier*, Peter Ralph‡, Axel Ullrich† &
Charles J. Sherr*

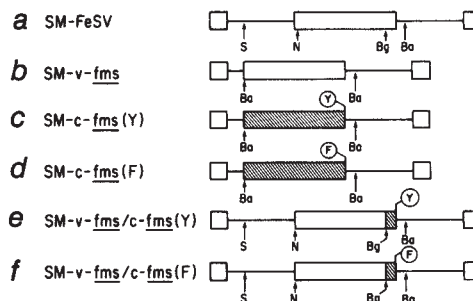
* Department of Tumor Cell Biology, St Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, Tennessee 38105, USA
† Department of Developmental Biology, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA
‡ Department of Cell Biology, Cetus Corporation, 1400 Fifty-third Street, Emeryville, California 94608, USA.

The *c-fms* proto-oncogene encodes a transmembrane glycoprotein that is probably identical to the receptor for the macrophage colony stimulating factor, CSF-1¹. Forty C-terminal amino acids of the normal receptor are replaced by 11 unrelated residues in the feline *v-fms* oncogene product², deleting a C-terminal tyrosine residue (Tyr⁹⁶⁹) whose phosphorylation might negatively regulate the receptor kinase activity³⁻⁵. We show that the human *c-fms* gene stimulates growth of mouse NIH 3T3 cells in agar in response to human recombinant CSF-1, indicating that receptor transduction is sufficient to induce a CSF-1 responsive phenotype. Although cells transfected with *c-fms* genes containing either Tyr⁹⁶⁹ or Phe⁹⁶⁹ were not transformed, cotransfection of these genes with CSF-1 complementary DNA induced transformation, with *c-fms*(Phe⁹⁶⁹) showing significantly more activity than *c-fms*(Tyr⁹⁶⁹). In the absence of CSF-1, chimaeric *v-fms/c-fms* genes encoding the wild-type *c-fms* C terminus were poorly transforming, whereas chimaeras bearing Phe⁹⁶⁹ were as transforming as *v-fms*. Thus, the Phe⁹⁶⁹ mutation, although not in itself sufficient to induce transformation, activates the oncogenic potential of *c-fms* in association with an endogenous ligand or in conjunction with mutations elsewhere in the *c-fms* gene that confer ligand-independent signals for growth.

Retroviral vectors were constructed that contained either human *c-fms* cDNA or chimaeric genes formed between the feline *v-fms* and human *c-fms* genes (Fig. 1). A *Bgl*II site shared by the 3' region of both genes was used to replace the 38 C-terminal amino acids of the *v-fms* gene product with the corresponding 67 amino acid *c-fms*-encoded C-terminus. Mutagenesis with a synthetic oligonucleotide was used to convert Tyr⁹⁶⁹ of the *c-fms* gene product to a phenylalanine residue, and both the mutated *c-fms* gene and a chimaeric *v-fms/c-fms* construct bearing this mutation were inserted into vectors. We used both the DNA provirus of the Susan McDonough strain of feline sarcoma virus (SM-FeSV)⁶ from which FeLV *gag* and all or part of the *v-fms* sequences were deleted, and a Moloney murine leukaemia virus (MuLV)-based vector, pZIP-neoSV(X)I⁷, that contains a neomycin resistance gene as a dominant selectable marker.

DNA constructs were transfected into NIH 3T3 cells and foci of transformed cells were counted three weeks later. The efficiency of focus formation obtained with the different constructs is listed in Table 1, and representative Giemsa-stained plates are shown in Fig. 2. The *v-fms* gene (Fig. 1a, g) efficiently stimulated focus formation^{6,8} generally yielding 150-300 foci per plate per 30 ng of input plasmid, corresponding to 5-9 × 10³ focus-forming units (f.f.u.) per µg (Table 1; Fig. 2a). Constructs

SM-FeSV Vectors:



pZIPneo Vectors:

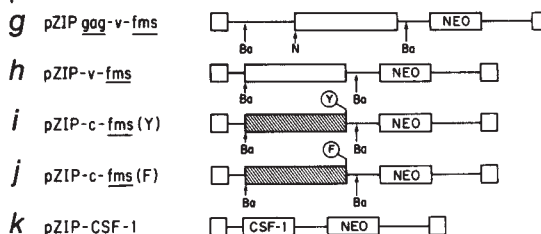
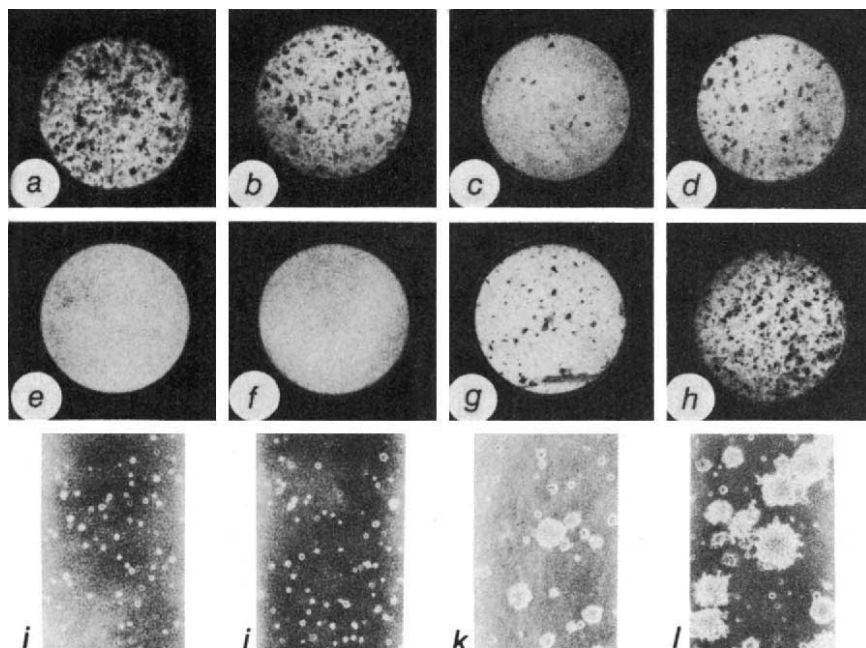


Fig. 1 Retroviral DNA constructs transfected into NIH 3T3 cells. The *v-fms*, *c-fms* and chimaeric *v-fms/c-fms* genes were inserted into a vector derived from the molecularly cloned SM-FeSV provirus (a)⁶. The vector was prepared by converting a *Sac*I site (S) 5' to the *gag* leader to a *Bam*HI site (Ba) using synthetic oligonucleotide linkers and excising the internal *Bam*HI fragment that contained *gag-v-fms* coding sequences⁹. c, d, Site-specific mutagenesis was used to convert Tyr⁹⁶⁹ of the *c-fms* gene (TAT) to Phe⁹⁶⁹ (TTC) and *Bam*HI fragments containing the wild-type and mutated *c-fms* alleles were inserted into the vector. e, f, Chimaeric *v-fms/c-fms* genes were constructed by codigestion of SM-FeSV DNA (a) with *Bgl*II (Bg) and *Bam*HI and substituting the homologous 3' regions of the *c-fms*(Tyr⁹⁶⁹) [Y] and *c-fms*(Phe⁹⁶⁹) [F] genes for *v-fms* sequences. The construction of pZIP-*gag-v-fms* (g) and pZIP-*v-fms* (h) has been described in detail previously⁹. i, j, The vectors were used to prepare pZIP-*c-fms*^Y and pZIP-*c-fms*^F by identical methods to those used for SM-FeSV-based vectors (c, d). The SM-FeSV *gag* sequences were eliminated by deletion of sequences between the *Sac*I site (S) 5' to the *gag* leader and an *Nco*I site (N) just 5' to an internal ATG codon in the *v-fms* gene; the latter ATG codon is homologous to the bona fide initiation codon of the *c-fms* gene, and 'gag-minus' constructs (h) have been found to exhibit full transforming activity⁷. To retain the nucleotide configuration around the initiation codon, we appended a palindromic linker sequence containing a site of restriction for *Bam*HI (Ba) just 5' to the *v-fms* *Nco*I site. A similar *gag*-minus derivative was prepared by excising the internal *Bam*HI fragment of pZIP-*v-fms* (h) and inserting it into the SM-FeSV-based vector (b). A CSF-1 cDNA¹⁴ was excised from an M13 clone with *Xho*I, treated with the Klenow fragment of DNA polymerase I, and inserted into the blunt-ended *Bam*HI cloning site of pZIPneoSV(X)I to give pZIP-CSF-1 (k). All constructs were in pBR322.

from which *gag* sequences were deleted (Fig. 1, and Table 1, B, H) transformed NIH 3T3 cells almost as efficiently as SM-FeSV (Fig. 2b)⁹. In contrast, vectors containing *c-fms* (Tyr⁹⁶⁹) (Table 1, C, I) and *c-fms* (Phe⁹⁶⁹) (D, J) did not yield foci (Fig. 2e, f). The chimaeric *v-fms/c-fms* (Tyr⁹⁶⁹) gene (Table 1, E) was poorly transforming (Fig. 2c), whereas *v-fms/c-fms* (Phe⁹⁶⁹) (Table 1, F) induced foci as efficiently as *v-fms* (Fig. 2d). The foci obtained with *v-fms/c-fms* (Phe⁹⁶⁹) were morphologically indistinguishable from those induced by *v-fms* itself and arose as rapidly, but those induced by *v-fms/c-fms* (Tyr⁹⁶⁹) grew much more slowly and were generally smaller. In each case, the number of foci was a linear function of DNA dose, suggesting that the information required for transformation resided within a single class of DNA molecules. As the results with two vector systems were similar, the biological activities of the various

Fig. 2 Focus- and colony-formation by transfected NIH 3T3 cells. DNA transfection assays were performed as described⁸ with the retroviral DNA constructs shown in Fig. 1. Foci of transfected cells were counted microscopically 3 weeks after transfection (see Table 1) and stained with Giemsa. Representative results with SM-FeSV-based vectors are shown for cells receiving 30 ng input plasmid plus 5 μ g carrier DNA. The stained plates contain cells transfected with: a, SM-FeSV; b, SM-v-*fms*; c, v-*fms*/c-*fms* (Tyr⁹⁶⁹); d, v-*fms*/c-*fms* (Phe⁹⁶⁹); e, c-*fms* (Tyr⁹⁶⁹); f, c-*fms* (Phe⁹⁶⁹); g, c-*fms* (Tyr⁹⁶⁹) plus CSF-1; h, c-*fms* (Phe⁹⁶⁹) plus CSF-1. NIH 3T3 cells transfected with c-*fms* (Tyr⁹⁶⁹) or c-*fms* (Phe⁹⁶⁹) were selected in G418 and seeded as single cells into semisolid medium. Cells containing c-*fms* (Tyr⁹⁶⁹) (i, k) or c-*fms* (Phe⁹⁶⁹) (j, l) were grown in the absence (i, j) or presence (k, l) of medium containing 2,000 units ml⁻¹ human recombinant CSF-1. Colonies were photographed 2 weeks after plating (400 $\times$ magnification).



constructs appears to be due to structural differences in the inserted genes.

Each of the SM-FeSV-based vectors was cotransfected into NIH 3T3 cells with a pSV2neo plasmid, and recipient cells were selected in medium containing G418¹⁰. Cells transfected with pZIPneo vectors were selected similarly. Each cell line was metabolically labelled with [³⁵S]methionine, and the radiolabelled *fms* gene products were precipitated with antisera and analysed on polyacrylamide gels. Cells transfected with vectors containing wild-type or mutant c-*fms* cDNAs, or with chimaeric v-*fms*/c-*fms* genes all expressed *fms*-coded proteins at equivalent levels (Fig. 3a, lanes 2–7). As predicted, the chimaeric v-*fms*/c-*fms*-coded molecules were slightly larger than their v-*fms*-coded counterparts. Immune complex kinase assays performed with an admixed amino-acid copolymer (poly Glu-Tyr) showed that all *fms* encoded polypeptides had an associated tyrosine kinase activity that phosphorylated both the *fms*-encoded proteins and the exogenous substrate (Fig. 3b). Under these conditions, no significant differences in kinase activity were detected between matched v-*fms*/c-*fms*- or c-*fms*-coded glycoproteins containing either Tyr⁹⁶⁹ or Phe⁹⁶⁹. This was not unexpected, as the reactions *in vitro*, although they afford an assay of the steady-state levels of the autophosphorylated proteins, may not accurately measure differences in enzyme specific activity. Based on these analyses, the fact that v-*fms* induces transformation, whereas both mutant and wild-type c-*fms* genes are inactive, must be due to structural differences between the transfected gene products and not their levels of synthesis. Moreover, the increased transforming activity of v-*fms*/c-*fms* (Phe⁹⁶⁹) compared to that of v-*fms*/c-*fms* (Tyr⁹⁶⁹) shows that the Tyr to Phe mutation potentiates transformation.

Because the v-*fms*/c-*fms* (Phe⁹⁶⁹) gene was efficiently transforming whereas c-*fms* (Phe⁹⁶⁹) was not, additional genetic alterations within the body of the v-*fms* gene must complement the C-terminal Phe⁹⁶⁹ mutation in transforming NIH 3T3 cells. The feline v-*fms* gene product is 84% homologous to the human c-*fms*-coded glycoprotein^{2,11}, so certain differences between the two genes could represent 'activating mutations'. Although the v-*fms* gene encodes the complete ligand-binding domain of the normal receptor^{2,9,12}, the v-*fms* kinase acts constitutively, transforming both fibroblasts and macrophages in the absence of CSF-1 (refs 12, 13). If mutations in the body of the v-*fms* gene are in part responsible for activating the receptor kinase in the absence of ligand, persistent ligand stimulation of the c-*fms*

Table 1 Efficiency of focus formation with different retroviral DNA constructs

Retroviral DNA construct		Focus forming units per μ g input DNA
A	SM-FeSV	8.8×10^3
B	SM-v- <i>fms</i>	4.2×10^3
C	SM-c- <i>fms</i> ^Y (Tyr ⁹⁶⁹)	<10
D	SM-c- <i>fms</i> ^F (Phe ⁹⁶⁹)	<10
E	SM-v- <i>fms</i> /c- <i>fms</i> ^Y (Tyr ⁹⁶⁹)	4.1×10^2
F	SM-v- <i>fms</i> /c- <i>fms</i> ^F (Phe ⁹⁶⁹)	4.3×10^3
G	pZIP-gag-v- <i>fms</i>	5.0×10^3
H	pZIP-v- <i>fms</i>	2.0×10^3
I	pZIP-c- <i>fms</i> ^Y (Tyr ⁹⁶⁹)	<10
J	pZIP-c- <i>fms</i> ^F (Phe ⁹⁶⁹)	<10
K	pZIP-CSF-1	<10
L	SM-c- <i>fms</i> ^Y + pZIP-CSF-1	9.0×10^2
M	SM-c- <i>fms</i> ^F + pZIP-CSF-1	7.5×10^3
N	pZIP-c- <i>fms</i> ^Y + pZIP-CSF-1	4.0×10^2
O	pZIP-c- <i>fms</i> ^F + pZIP-CSF-1	6.9×10^3
P	SM-v- <i>fms</i> /c- <i>fms</i> ^Y + pZIP-CSF-1	3.0×10^2
Q	SM-v- <i>fms</i> /c- <i>fms</i> ^F + pZIP-CSF-1	3.1×10^3

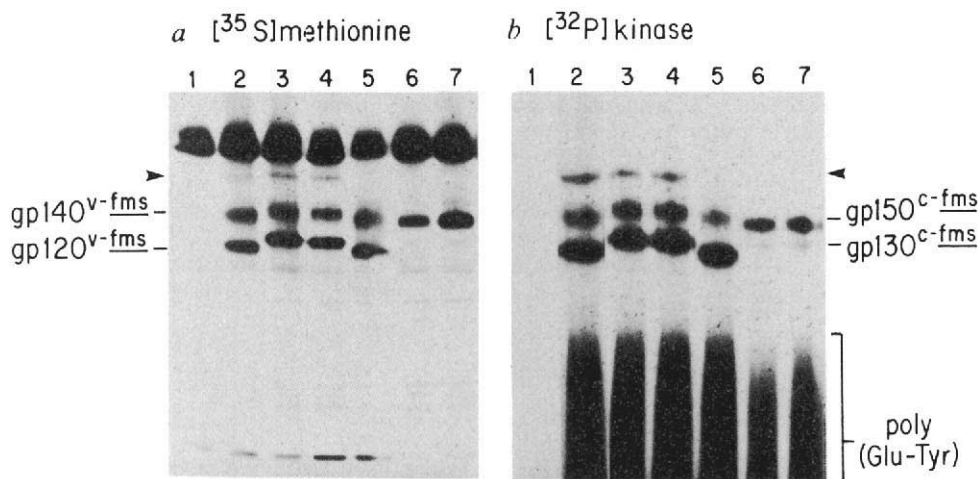
Plasmid DNAs containing the retroviral constructs shown in Fig. 1 were transfected as described⁸ into NIH 3T3 cells by the calcium phosphate procedure; cultures were split 1:3 one day after transfection, fed every 3 days, and foci of transformed cells (see Fig. 2) were counted using a microscope 3 weeks after transfection. The number of foci was a linear function of DNA concentration. Averages from three independent experiments are given. In some experiments (L–Q), two different constructs were co-transfected in a 1:1 molar ratio, and the efficiency of focus formation was calculated from the total DNA input. SM-FeSV-based retroviral DNA constructs were cotransfected in parallel with pSV2neo, and recipient cells were selected in medium containing G418 (ref. 10). Cells containing pZIPneo vectors were directly selected in G418-containing medium. Cell lines transfected with pZIP-CSF-1 alone, or together with other retroviral vectors, were selected similarly and were shown to secrete the growth factor at levels of 1,000–2,000 units per ml of medium, as assayed biologically with the CSF-1-dependent murine BAC1.2F5 macrophage cell line¹². Constructs A–K are shown in Fig. 1a–k.

gene product might also elicit a transformed phenotype.

We therefore cotransfected either the wild-type or mutant c-*fms* genes together with a second retroviral construct containing a biologically active, human CSF-1 cDNA¹⁴ (Fig. 1k) into NIH 3T3 cells. Bioassays showed that the transfected cells produced 1,000–2,000 units ml⁻¹ of growth factor when titred on the murine BAC1.2F5 macrophage cell line¹². Cotransfection of c-*fms* (Tyr⁹⁶⁹) with CSF-1 cDNA yielded transformed foci (Table 1, L, N; Fig. 2g), but c-*fms* (Phe⁹⁶⁹) was considerably more active in the cotransfection assay (Table 1, M, O; Fig. 2h).

Fig. 3 *a*, Synthesis and *b*, tyrosine kinase activities of *fms*-encoded proteins in transfected NIH 3T3 cells. Representative results with SM-FeSV-based vectors are shown. In each panel, the lanes are ordered as follows: uninfected NIH 3T3 cells (lane 1); cells transfected with SM-FeSV (lane 2); *v-fms/c-fms*(Tyr⁹⁶⁹) (lane 3); *v-fms/c-fms*(Phe⁹⁶⁹) (lane 4); *v-fms* (*gag*-minus) (lane 5); *c-fms*(Tyr⁹⁶⁹) (lane 6); and *c-fms*(Phe⁹⁶⁹) (lane 7). The mobilities of the immature (gp120^{v-fms}) and mature cell surface form (gp140^{v-fms}) of the *v-fms*-encoded glycoproteins are indicated in the left margin, and the positions of the analogous *c-fms*-coded glycoproteins (gp130^{c-fms} and gp150^{c-fms}) appear in the right margin. The immature forms of the *v-fms* and *c-fms* glycoproteins differ from the mature cell surface forms only in their composition of *N*-linked oligosaccharide chains^{22,24}. Because *gag* sequences were retained in constructs containing chimaeric *v-fms/c-fms* molecules (Fig. 1), the two genes specify *gag-fms* fusion proteins (arrows in margins) that undergo cotranslational cleavage of the *gag*-coded fragment to generate glycoproteins analogous to gp120^{v-fms} (refs 6, 9, 22). The polypeptides encoded by the *v-fms/c-fms* chimaeric genes are longer than those specified by *v-fms* due to the presence of additional C-terminal amino acids.

Methods. *a*, Cells were metabolically labelled with [³⁵S]methionine for 90 minutes²² then lysed in RIPA buffer (50 mM Tris HCl, pH 7.4, containing 150 mM NaCl, 20 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% sodium dodecyl sulphate) with 2% Aprotinin and 1 mM phenylmethylsulphonyl fluoride as protease inhibitors. *b*, Unlabelled cell lysates for protein kinase assays were prepared in parallel. Lysates were cleared of nuclei by low-speed centrifugation and incubated with antiserum to a recombinant *v-fms*-coded polypeptide expressed in bacteria²³ that reacts with the *v-fms*- and human *c-fms*-coded gene products^{1,24}. Immune complexes containing metabolically labelled polypeptides were recovered with *Staphylococcus aureus* protein-A-Sepharose, denatured and separated electrophoretically on 6% polyacrylamide gels containing SDS²². Unlabelled immune complexes were washed in RIPA buffer and suspended in a 10 µl reaction mix containing 50 mM HEPES buffer, pH 7.4, 10 mM MnCl₂, 1% Triton X-100, and 20 µM [^γ-³²P]ATP containing 2 mg ml⁻¹ poly(Glu⁸⁰:Tyr²⁰)_n with an average relative molecular mass of 43,000²⁵ (Sigma). After incubation for 10 min at 30 °C, the precipitates were analysed on gels as described above. The radiolabelled products were identified after fluorography (*a*) or autoradiography (*b*). The results in each panel are derived from quantitative immunoprecipitation of lysates from the same number of cells, labelled in parallel. Exposure times of the autoradiograms are 12 h (*a*) and 1 h (*b*). Each panel shows a composite of lanes from the same gel.



Cotransfection of the CSF-1 gene together with *v-fms/c-fms* chimaeric molecules did not augment their transforming efficiency (Table 1, P, Q) and no foci were obtained with the CSF-1 gene alone (Table 1, K). Thus, persistent stimulation of the normal *c-fms*-encoded receptor by the product of a transduced human CSF-1 gene led to transformation, and the transforming efficiency was increased to that of the viral oncogene by the Phe⁹⁶⁹ mutation.

G418-selected cell lines transformed by *v-fms* or by *v-fms/c-fms*(Phe⁹⁶⁹) formed tumours in nude mice within 2 weeks (5 × 10⁶ cells per animal), whereas cells expressing *v-fms/c-fms*(Tyr⁹⁶⁹) or *c-fms*(Phe⁹⁶⁹) plus CSF-1 formed tumours within 4 weeks. Cells cotransfected with *c-fms*(Tyr⁹⁶⁹) and the CSF-1 gene induced tumours after a longer latency period (6–9 weeks), but cells expressing *c-fms*(Tyr⁹⁶⁹) or *c-fms*(Phe⁹⁶⁹) did not induce tumours by 14 weeks. Explanted tumour cells were readily established in culture and expressed *fms*-encoded glycoproteins. Thus, transformation *in vitro* correlated with tumorigenicity *in vivo*.

Because human *c-fms* cDNA stimulated fibroblast transformation when it was coexpressed with the human CSF-1 gene, NIH 3T3 cells transfected with *c-fms* alone should respond to exogenous CSF-1. Cells expressing wild-type *c-fms*(Tyr⁹⁶⁹) or mutant *c-fms*(Phe⁹⁶⁹) were cloned in semisolid medium in the presence or absence of 2,000 units ml⁻¹ of recombinant human CSF-1. In the absence of the growth factor, no colonies were observed (plating efficiency <0.002%), but both cell lines formed colonies when human CSF-1 was added (plating efficiency ~15%) (Fig. 2i–l). Although the colonies obtained with cells expressing *c-fms*(Phe⁹⁶⁹) (Fig. 2l) were generally larger than those expressing *c-fms*(Tyr⁹⁶⁹) (Fig. 2k), no differences were detected in the numbers of colonies or in their dose dependence on CSF-1. In contrast, the *v-fms/c-fms* chimaeric constructs, and cells cotransfected with *c-fms*(Tyr⁹⁶⁹) or *c-fms*(Phe⁹⁶⁹) together with CSF-1 DNA, stimulated the formation of NIH 3T3 agar colonies in the absence of exogenous CSF-1 (plating efficiency ~20%), and addition of CSF-1 had no further effect on cell growth. These results formally demonstrate that

the human *c-fms* proto-oncogene encodes a functional CSF-1 receptor and indicate that this receptor, normally expressed by mononuclear phagocytes, can also stimulate ligand-induced proliferation of fibroblasts. The susceptibility of transfected fibroblasts to CSF-1 implies that physiological substrates for the receptor kinase are coexpressed in these cells.

Neither the wild-type nor mutated *c-fms* allele was active by itself as a transforming gene. Similar results were obtained with a *c-fms* gene bearing a truncated 3' coding sequence analogous to that of *v-fms* (data not shown), indicating that these structural alterations are insufficient for transformation. NIH 3T3 cells transfected with the *v-sis* oncogene encoding the B chain of platelet-derived growth factor (PDGF) coexpress PDGF receptors and undergo transformation by an autocrine mechanism^{15,16}. Similarly, cells transfected with the wild-type *c-fms* allele together with human CSF-1 cDNA underwent transformation, and the effect of the Phe⁹⁶⁹ mutation was unmasked when the mutated receptor was coexpressed with the CSF-1 gene. Thus, a critical alteration at the *c-fms* C terminus can potentiate transformation in the presence of an endogenous ligand. An analogous result was obtained with *v-fms/c-fms* chimaeric constructs in which substitution of *v-fms* for *c-fms* sequences bypassed the requirement for a CSF-1 gene in transformation. The *v-fms/c-fms*(Phe⁹⁶⁹) gene was considerably more efficient than *v-fms/c-fms*(Tyr⁹⁶⁹), yielding larger and more frequent foci when tested in parallel. The simplest interpretation is that activation of the *c-fms* proto-oncogene to an efficiently transforming oncogene involves two modifications: one that alters the C terminus, and a second that simulates a ligand-induced conformational change, rendering the receptor kinase CSF-1-independent.

The effects of the mutation at Tyr⁹⁶⁹ suggest that this residue might serve as a regulatory phosphorylation site in the *c-fms* gene product. The position of this residue is analogous to that of Tyr⁵²⁷ of pp60^{c-src} which is phosphorylated *in vivo*³, may function in negatively regulating *c-src* kinase activity^{3,5}, and was deleted from the C terminus of the transforming Rous sarcoma virus *v-src* gene product¹⁷. Analogous sites of tyrosine

phosphorylation are also present within the receptor for epidermal growth factor but were deleted in the formation of the *v-erb-B* oncogene^{18,19}. Although several different mutations within the body of the *c-src* gene can activate its transforming activity^{4,20,21}, all the mutant transforming *c-src* proteins lacked phosphate residues at Tyr⁵²⁷ when expressed in transformed cells⁴. Similar alterations within the *v-fms* gene are physiologically significant and potentiate its transforming activity, presumably by a similar mechanism.

We thank Dr E. Kawasaki (Cetus Corporation) for CSF-1 cDNA and Dr S. Clark (Genetics Institute, Cambridge,

Massachusetts) for human recombinant CSF-1 and Mr Virgil Holder and Ms Shawn Kramer for technical assistance. This work was supported in part by grant CA 38187 from the National Cancer Institute, NIH (to C.J.S.) and by the American Lebanese Syrian Associated Charities of St Jude Children's Research Hospital. C.W.R. was supported by a Biomedical Research Support Grant (RR-05584) from NIH.

Note added in proof: Browning *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **83**, 7800-7804) recently demonstrated that a chimaeric DNA construct similar to that shown in Fig. 1e was attenuated in transforming activity.

Received 25 September; accepted 12 November 1986.

- Sherr, C. J. *et al.* *Cell* **41**, 665-676 (1985).
- Coussens, L. *et al.* *Nature* **320**, 277-280 (1986).
- Cooper, J. A., Gould, K. L., Cartwright, C. A. & Hunter, T. *Science* **231**, 1431-1434 (1986).
- Iba, H., Cross, F., Garber, E. A. & Hanafusa, H. *Molec. cell. Biol.* **5**, 1058-1066 (1985).
- Courtneidge, S. A. *EMBO J.* **4**, 1471-1477 (1985).
- Donner, L., Fedele, L. A., Garon, C. F., Anderson, S. J. & Sherr, C. J. *J. Virol.* **41**, 489-500 (1982).
- Cepko, C. L., Roberts, B. E. & Mulligan, R. C. *Cell* **37**, 1053-1062 (1984).
- Roussel, M. F., Rettenmier, C. W., Look, A. T. & Sherr, C. J. *Molec. cell. Biol.* **4**, 1999-2009 (1984).
- Wheeler, E. F. *et al.* *J. Virol.* **59**, 224-233 (1986).
- Southern, P. J. & Berg, P. *J. molec. appl. Genet.* **1**, 327-338 (1982).
- Hampe, A., Gobet, M., Sherr, C. J. & Galibert, F. *Proc. natn. Acad. Sci. U.S.A.* **81**, 85-89 (1984).

- Sacca, R., Stanley, E. R., Sherr, C. J. & Rettenmier, C. W. *Proc. natn. Acad. Sci. U.S.A.* **83**, 3331-3335 (1986).
- Wheeler, E. F., Rettenmier, C. W., Look, A. T. & Sherr, C. J. *Nature* **324**, 377-380 (1986).
- Kawasaki, E. S. *et al.* *Science* **230**, 291-296 (1985).
- Waterfield, M. D. *et al.* *Nature* **304**, 35-39 (1983).
- Doolittle, R. F. *et al.* *Science* **221**, 275-277 (1983).
- Takeya, T. & Hanafusa, H. *Cell* **32**, 881-890 (1983).
- Ullrich, A. *et al.* *Nature* **309**, 418-425 (1984).
- Downward, J., Parker, P. & Waterfield, M. D. *Nature* **311**, 483-485 (1984).
- Iba, H., Takeya, T., Cross, F. R., Hanafusa, T. & Hanafusa, H. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4424-4428 (1984).
- Levy, J. B., Iba, H. & Hanafusa, H. *Proc. natn. Acad. Sci. U.S.A.* **83**, 4228-4232 (1986).
- Anderson, S. J., Gonda, M. A., Rettenmier, C. W. & Sherr, C. J. *J. Virol.* **51**, 730-741 (1984).
- Furman, W. L. *et al.* *Virology* **152**, 432-445 (1986).
- Rettenmier, C. W. *et al.* *J. clin. Invest.* **77**, 1740-1746 (1986).
- Braun, S., Raymond, W. E. & Racker, E. *J. biol. Chem.* **259**, 2051-2054 (1984).

Abelson-transformed fibroblasts contain nuclear phosphotyrosyl-proteins which preferentially bind to murine DNA

John C. Bell*§, Louis C. Mahadevan*, William H. Colledge*, A. Raymond Frackelton Jr†, Michael G. Sargent‡ & J. Gordon Foulkes*||

* Laboratory of Eukaryotic Molecular Genetics and † Laboratory of Embryogenesis, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK

‡ Department of Medicine, Brown University, Roger Williams General Hospital, 825 Chalkstone Avenue, Providence, Rhode Island 02908, USA

Protein-tyrosine kinases, either in the form of growth-factor receptors¹⁻³ or as the polypeptide products of oncogenes^{4,5}, appear to be important in the regulation of cell growth and transformation. A major question, however, is how their substrates mediate changes in gene expression (reviewed in refs 6-8). Because binding of proteins to specific DNA sequences represents the most direct mechanism for regulating transcription, we have investigated the possibility that some DNA-binding proteins may be substrates of protein-tyrosine kinases. Here, we present evidence for nuclear phosphotyrosyl-proteins in murine fibroblasts transformed by the *v-abl* protein-tyrosine kinase. Furthermore, we have found that these proteins are not significantly phosphorylated in normal NIH 3T3 cells. Finally, using affinity competition chromatography with bacterial and mouse DNA, we have demonstrated that some of these proteins preferentially bind to mouse DNA. The identification of phosphotyrosyl-proteins with selective DNA-binding properties suggests a possible mechanism through which protein-tyrosine kinases may effect changes in gene transcription.

Normal and *v-abl*-transformed NIH 3T3 cells⁹, labelled with ³²P-orthophosphate, were fractionated by conventional methods (see Fig. 1 legend) into a cytoplasmic fraction and a nuclear pellet. These preparations showed a minimum of cross-contamination by a variety of biochemical markers (Table 1). The cytoplasmic fraction was applied directly to a column to which

an antibody which specifically recognizes phosphotyrosine¹⁰ had been covalently coupled. The nuclear pellet was first extracted with 2.5 M NaCl, clarified at 200,000g and then dialysed before immunoaffinity purification of solubilized nuclear proteins. Under these conditions, the cytoplasmic fraction from normal NIH 3T3 cells contained only a small amount of radioactive material which bound to the anti-phosphotyrosine column, consistent with the low levels of phosphotyrosine present in untransformed cells^{7,8,10}. We were unable to detect binding of NIH 3T3 nuclear proteins to the anti-phosphotyrosine column even after prolonged exposure of the autoradiograph (data not shown). In contrast, a number of phosphoproteins were immunopurified from both cytoplasmic and nuclear fractions derived from *v-abl*-transformed cells. Routinely, we found 8-10 times as much radioactive material immunoprecipitated from the cytoplasmic fraction as from the nuclear fraction. To allow a qualitative comparison, approximately equal amounts of radioactivity from the two fractions were resolved by two-dimensional gel electrophoresis (Fig. 1). Phospho-amino-acid analysis of 16 individual spots (Fig. 1, spots a-p) revealed that each phosphoprotein purified by this procedure was phosphorylated primarily on tyrosine (data not shown). Certain phosphoproteins from both fractions (compare spots b and j, Fig. 1A and B) appear to have identical electrophoretic mobilities, whereas others are unique to one or the other fraction (for example spots k and o, Fig. 1B). The oncogene-encoded transforming protein p120^{gag-abl} is recognized as a phosphotyrosine-containing protein in these immunoprecipitates¹⁰, but it is not well resolved on two-dimensional gels. Using antibodies specific for this protein, a significant fraction (≤15%) of p120^{gag-abl} and its associated tyrosine kinase activity is detectable in the nuclear fraction.

A proportion of authentic nuclear proteins would be expected to have DNA-binding activity and possibly to show sequence specificity. This possibility was tested by assaying the ability of nuclear phosphotyrosyl-proteins isolated from *v-abl*-transformed cells to bind to both homologous and heterologous double-stranded DNA *in vitro*, using a modification of the affinity-competition technique described by Sanzo *et al.*¹¹. A radioactive nuclear extract was circulated between a large column of heterologous (*Bacillus subtilis*) and a smaller column of homologous DNA (mouse) in a buffer containing 2.5 M NaCl. The nuclear extract was recirculated between the columns overnight at 2 °C while the ionic strength of the buffer was gradually

§ Present address: Department of Biochemistry, McGill University, 3655 Drummond Street, Montreal, Quebec, Canada H36 1Y6.

|| To whom correspondence should be addressed.

Table 1 Distribution of biochemical markers in fractionated transformed cells

Marker	Supernatant (%)	Nuclear pellet (%)	Cell fraction
Lactate dehydrogenase	99	1	Cytoplasm ^{*21}
5' Nucleotidase	99	1	Plasma membrane ²²
NADH cytochrome <i>c</i> reductase	98	2	Endoplasmic reticulum ²³
UDP galactosyl transferase	96	4	Golgi ²⁴
Cytochrome oxidase	84	16	Mitochondria ²⁵
Topoisomerase I	5	95	Nucleus ¹⁷
DNA	6	94	Nucleus ²⁶

* Sigma diagnostic kit 500.

The values presented are the average of at least three separate preparations and represent the per cent relative specific activities.

Fig. 1 Two-dimensional gel electrophoresis of immunoaffinity-purified phosphotyrosine-containing proteins. **A**, Immunoaffinity-purified cytoplasmic proteins; **B**, immunoaffinity-purified high-salt-solubilized nuclear proteins (autoradiography 5 days). The proteins labelled a-p were excised from the respective gels, acid hydrolysed, resolved by two-dimensional thin layer electrophoresis as described²⁷, and shown to be phosphorylated primarily on tyrosine residues. Relative molecular mass markers (in thousands) are shown to the left.

Methods. ANN-1 cells (10^7 in 5 ml phosphate-free DME of a p120^{gag-abl}-transformed NIH 3T3 cell line⁹) were labelled for 4 h in ^{32}P -orthophosphate (1 mCi ml^{-1}) and then lysed for 20 min on ice in nuclear lysis buffer (10 mM Tris-HCl pH 7.5, 10 mM NaCl, 3 mM Mg₂Cl, 0.1% NP-40, 30% sucrose, 500 μM sodium vanadate, 2 mM phenylmethylsulphonylfluoride (PMSF) aprotinin (10,000 units ml^{-1}) with occasional vortexing. The lysate was layered over nuclear lysis buffer containing 35% sucrose and nuclei pelleted by centrifugation (1,500g; 20 min). The cytoplasmic supernatant, including the 35% cushion, was passed through a Sephadex G-50 column (equilibrated with 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 500 μM sodium orthovanadate, 2 mM PMSF 1% Triton X-100) to remove nucleotides and applied to an anti-phosphotyrosine column containing 15 mg ml^{-1} of monoclonal antibody MA-2G8 (ref. 10). The nuclear pellet was resuspended in nuclear lysis buffer containing 1% Triton X-100 and layered over a cushion containing nuclear lysis buffer, 1% Triton X-100 and 35% sucrose. Nuclei were again pelleted, resuspended in a high salt buffer (2.5 M NaCl, 10 mM Tris-HCl pH 7.4, 5 mM EDTA, 1% Triton X-100, 500 μM sodium orthovanadate, 2 mM PMSF), extracted on ice for 20 min and then centrifuged (200,000g; 60 min). The high salt supernatant was passed through a Sephadex G-50 column and immunoaffinity purified as described above. After elution of phosphotyrosine-containing proteins from the columns with the phosphotyrosine analogue phenyl phosphate, the samples were concentrated by trichloroacetic acid (10% final) precipitation. Samples were resolved by isoelectric focusing in the first dimension and SDS-polyacrylamide gel electrophoresis in the second²⁸.

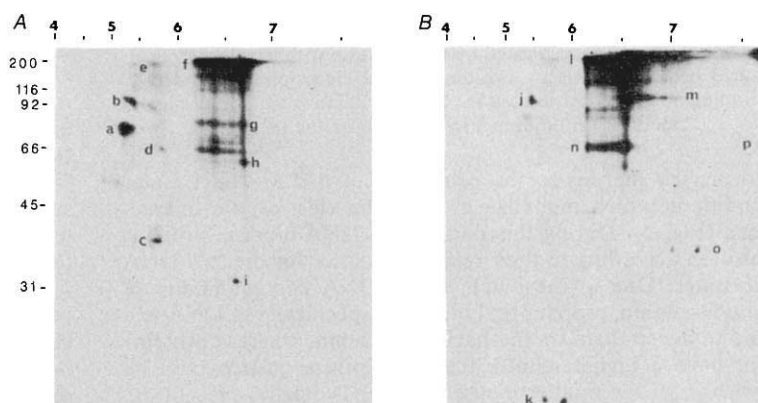
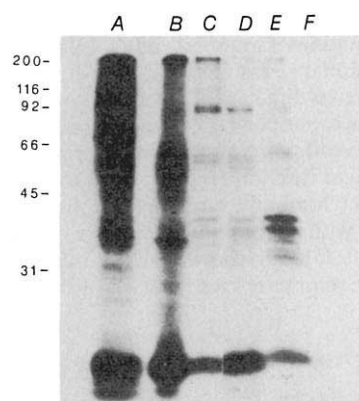


Fig. 2 DNA-binding activity of nuclear phosphotyrosyl-proteins. **A**, Nuclear phosphotyrosyl-proteins before fractionation on DNA columns. **B-E**, Phosphoproteins bound to: **B**, the bacterial column after one cycle; **C**, the mouse column after one cycle; **D**, the bacterial column after two cycles; **E**, the mouse column after two cycles. **F**, High salt wash of a Sephadex control column after two cycles. (Relative molecular mass markers, in thousands, are shown on the left.)

Methods. Nuclear phosphotyrosyl-proteins were prepared as described in Fig. 1 with the omission of the final 1% Triton X-100 wash and then made 2 M with respect to NaCl before introduction into the DNA-binding column circuit. The columns were equilibrated with 2 M NaCl, 1 mM sodium pyrophosphate, 5 mM EDTA, 0.02% polyvinyl pyrrolidone (relative molecular mass 10,000), 0.1 mM sodium orthovanadate, 0.1% Brij 35, 0.1% sodium deoxycholate, pH 8.0. The DNA columns were made from either *B. subtilis* or total mouse genomic DNA bound to Pharmacia DNA grade Sephadex G-25¹¹. In early experiments, we used standard fine grade Sephadex G-25 and found a significant amount of non-specific binding of phosphoproteins to the matrix itself, which was substantially reduced by using DNA grade G-25 Sephadex. The samples were circulated with intermittent pumping (30 min, 80 ml h^{-1} followed by 15 min without pumping) for 16 h at 2°C with gradual dialysis against column buffer to a final ionic strength equivalent to 0.05 M NaCl. At this stage, the columns were uncoupled and washed extensively with dialysis buffer before elution with 2.5 M NaCl. Typically, the circuit was composed of three control columns (Sephadex only) arranged alternately with columns of bacterial and mouse DNA. The order of the columns in the circuit neither qualitatively nor quantitatively affected binding of the proteins to the individual columns (not shown). Confirmation of the efficacy of this method in isolating murine DNA-binding proteins was obtained by recirculating the mouse DNA column eluates (the proteins in lane C) through the circuit of columns for a second cycle. Proteins which bound to the *Bacillus* and mouse DNA as well as to control Sephadex columns in the second cycle are shown. In the experiments shown here, the mouse column contained 50 μg of DNA whereas the bacterial column contained 2 mg of DNA.



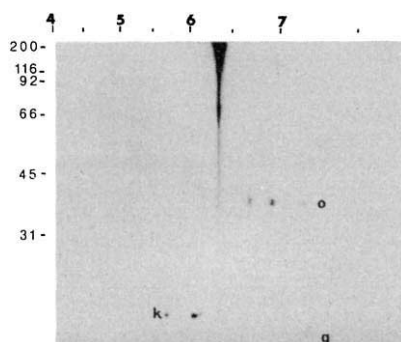


Fig. 3 Two-dimensional electrophoresis of mouse DNA-binding proteins. Nuclear phosphotyrosyl-proteins were subjected to one cycle of DNA binding (as described in Fig. 2) and the proteins that bound to the mouse column were eluted with 2.5 M NaCl. The eluate was concentrated by precipitation with trichloroacetic acid before analysis by two-dimensional electrophoresis. Autoradiography was carried out for 10 days. Relative molecular mass markers (in thousands) are shown on the left.

reduced by dialysis to the equivalent of 0.05 M NaCl under conditions which minimize non-specific electrostatic interactions (Fig. 2). During this period any DNA-binding proteins partition according to their relative affinities for the two DNA substrates. Due to the much higher DNA content of the *B. subtilis* column, proteins that bind non-specifically to DNA will tend to accumulate on the bacterial column, whereas proteins that have a higher affinity for eukaryotic sequences will be enriched on the smaller mouse column. Typically, 14% of the radioactive material loaded into the system bound to DNA with 12% on the *B. subtilis* column and 2% on the mouse column. For purposes of comparison, however, approximately equal amounts of material were loaded in each lane (Fig. 2). Clearly, a discrete group of phosphotyrosyl-proteins bound preferentially to the mouse DNA column (compare lanes B and C). To confirm this selectivity, phosphoproteins eluted from mouse DNA were recirculated through the columns under identical conditions, and the protein profiles after the second cycle are shown in lanes D, E and F (Fig. 2). In the second cycle, approximately equal amounts of radioactive material bound to the two DNA columns, although the *B. subtilis* DNA was in 40-fold excess over mouse DNA (Fig. 2). It is very likely that the specificity of the DNA-binding proteins for particular sequences is considerably greater than indicated by these experiments as the presentation of immobilized DNA is not optimized with respect to torsional stress or possible co-operative factors.

Figure 3 shows a two-dimensional electrophoretic analysis of the proteins that are enriched on the mouse DNA column. Compared to the overall pattern of phosphotyrosine-containing proteins in the nuclear fraction (Fig. 1), two groups of proteins (k and o) showed high-affinity binding to mouse DNA. These proteins co-migrate with a group of phosphotyrosine-containing proteins found in the solubilized nuclear material but not in the cytoplasmic fraction (compare Figs 1 and 3). In addition, protein

q (Fig. 3) although not clearly visible in the unfractionated nuclear material (Fig. 1 panel B) appears to be enriched after passage through the DNA-binding column. When cytoplasmic phosphotyrosyl-proteins were passed through the DNA columns, less than 0.05% of the ^{32}P -labelled phosphotyrosyl-proteins bound to DNA (compared to 14% after circulation of the nuclear fraction). No new DNA-binding proteins could be clearly resolved. Only a small amount (<10%) of the proteins designated as group k and none of the proteins in group o were detected (data not shown). These results confirmed the clear separation between the cytoplasmic and nuclear fractions as well as the specificity of the DNA-binding method we have employed. Not all the polypeptides found in the one-dimensional analysis (lane C, Fig. 2) were easily identified by two-dimensional electrophoresis. Possibly the more basic and acidic proteins are lost at the isoelectric focusing step but others may represent families of variably charged polypeptides thus diluting the radioactive signal on a two-dimensional gel.

Several groups have speculated upon the possible importance of nuclear phosphotyrosyl-proteins in pathways regulating gene expression¹²⁻¹⁶. The enzyme topoisomerase I was reported to be phosphorylated by pp60^{src} *in vitro*¹² but this does not seem to have any physiological role¹⁷. *In vitro* experiments with steroid receptors and purified kinases suggests that phosphorylation on tyrosine residues may be crucial for hormone-receptor interaction, with chromatin^{15,16}. More recently, the phosphorylation of steroid receptors on tyrosine residues *in vivo* has been reported¹⁸. Finally, the internalization of growth-factor-receptor tyrosine kinases to a juxtanuclear position after hormone stimulation¹⁹ and a similar localization of a subset of v-src and c-src gene products²⁰ suggests that signalling pathways for protein-tyrosine kinases could involve nuclear substrates. Although a significant fraction of p120^{gag-abl} protein and tyrosine kinase activity is detectable in the nuclear fraction, it is not clear whether this kinase phosphorylates the nuclear substrates, or if phosphorylation occurs before or after these substrates enter the nucleus.

At present, we are unable to determine what role tyrosine phosphorylation plays in regulating the biological activity of the DNA-binding proteins or indeed what genes they may regulate. It is possible that tyrosine phosphorylation could be involved in the transport of DNA-binding proteins to the nucleus, in the regulation of their catalytic activity, or perhaps modulate their binding specificity. It is tempting to speculate that they are involved in the control of genes that determine the transformed state. In support of this idea, we have not detected phosphorylation of these proteins in untransformed NIH 3T3 fibroblasts. Regardless of their exact role, the identification of this family of phosphotyrosyl-proteins with affinity for double-stranded mouse DNA, suggests a new element in the networks which control cell growth and transformation.

This work was supported by the MRC of the UK. J.C.B. was supported by a Canadian NCI post-doctoral fellowship. A portion of this work was supported by a PHS grant to A.R.F. from the NCI, grant CA 39235. W.H.C. holds a joint MRC-Imperial Chemical Industries PLC, PhD partnership award.

Received 4 August; accepted 11 December 1986.

- Ushiro, H. & Cohen, S. *J. biol. Chem.* **255**, 8363-8365 (1980).
- Ek, B., Westermarck, B., Westesson, A. & Heldin, C. H. *Nature* **295**, 419-420 (1982).
- Scher, C. J. *et al. Cell* **41**, 665-676 (1985).
- Hunter, T. & Sefton, B. M. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1311-1315 (1980).
- Witte, O. N., Dasgupta, A. & Baltimore D. *Nature* **283**, 826-831 (1980).
- Zullo, J. N., Cochran, B. H., Huang, A. S. & Stiles C. D. *Cell* **43**, 793-800 (1985).
- Hunter, T. & Cooper, J. A. *Rev. Biochem.* **54**, 897-930 (1985).
- Foulkes, J. G. & Rich Rosner, M. in *Molecular Aspects of Cellular Regulation 4* (eds Cohen, P. & Houslay, M.) 217-252 (Elsevier, Amsterdam, 1985).
- Scher, C. D. & Siegler, R. *Nature* **253**, 729-731 (1975).
- Frackelton, A. R., Ross, A. H. & Eisen, N. N. *Molec. Cell Biol.* **3**, 3173-3178 (1984).
- Sanzo, M., Stevens, B., Tsai, M. & O'Malley, B. W. *Biochemistry* **23**, 6491-6498 (1984).
- Tse-Dinh, Y. C., Wong, T. W. & Goldberg, A. R. *Nature* **312**, 785-786 (1984).
- Blat, C., Harel, L., Villaudy, J. & Golde, A. *Expl Cell Res.* **134**, 121-128 (1981).
- Blat, C., Harel, L., Villaudy, J. & Golde, A. *Expl Cell Res.* **145**, 305-314 (1983).

- Ghosh-Dastidar, P., Coty, W. A., Griest, R. E., Woo, D. D. L. & Fox, C. F. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1645-1658 (1984).
- Migliaccio, A., Rotondi, A. & Auricchio, F. *Proc. natn. Acad. Sci. U.S.A.* **81**, 5921-5925 (1984).
- Colledge, W. H., Edge, M. & Foulkes, J. G. *Biosc. Rep.* **6**, 301-307 (1986).
- Migliaccio, A., Rotondi, A. & Auricchio, F. *EMBO J.* **5**, 2867-2872 (1986).
- Miller, K., Beardmore, J., Kanety, H., Schlessinger, J. & Hopkins, C. R. *J. Cell Biol.* **102**, 500-509 (1986).
- Resh, M. D. & Erikson, R. L. *J. Cell Biol.* **100**, 409-417 (1985).
- Cabaud, P. G. & Wroblewski, F. *Am. J. clin. Path.* **30**, 234 (1958).
- Newby, A. C., Luzio, J. P. & Hales, C. N. *Biochem. J.* **146**, 625-633 (1975).
- Beufay, H. *et al. J. Cell Biol.* **61**, 213-231 (1974).
- Bretz, R. & Staubli, W. *Eur. J. Biochem.* **77**, 181-192 (1977).
- Cooperstein, S. J. & Lazarow, A. *J. biol. Chem.* **189**, 665-670 (1951).
- Labarca, C. & Paigen, K. *Analyt. Biochem.* **102**, 344-352 (1980).
- Heath, J. K., Mahadevan, L. & Foulkes, J. G. *EMBO J.* **5**, 1809-1814 (1986).
- O'Farrell, P. H. *J. biol. Chem.* **250**, 4007-4021 (1975).

later date. Bulletin boards are available for requesting reagents, for discussing scientific topics such as gene expression, oncogenes, plant molecular biology, or molecular evolution, and for obtaining information about PC's and solutions to technical computer problems.

The advent of personal computers and PC software for sequence analysis has shifted many of the simpler tasks from mainframes to these smaller machines. Files created with PC programs, such as Beckman Instruments' MicroGenie and IntelliGenetics' PC/GENE, can be transferred by phone and used on the BIONET mainframe computer. PC/GENE automatically converts sequence files to the BIONET format, and transmits them to the BIONET mainframe.

Future applications

The usefulness of the BIONET resource has been reflected in its steady growth. Sixteen Telenet ports and six direct dial ports are now available for simultaneous access. The Telenet ports can be reached by a local phone call from many locations around the world. Unfortunately, the demand for time on the system has reached the point where it is causing a slowdown in program response time during peak usage hours. To alleviate this problem, IntelliGenetics is upgrading its current computer hardware, and licensing its software for use at other locations on VAX mini-computers, Sun workstations, and DEC 2060's. These outside computing facilities are termed "BIONET satellites" and are provided with access to BIONET electronic mail and bulletin board services. The satellite computers reduce the load on the main BIONET computer while maintaining communication links between all BIONET users.

In the future, expansion of this network should promote greater exchange of information between members of the scientific community. The possibility of holding continuous scientific meetings through BIONET's electronic bulletin boards has advantages that warrant consideration, and this application may not be too far off. The network has already encouraged international collaborations among researchers, and this trend should accelerate as an increasing number of scientists around the world learn about the system and acquire computer skills. □

For further information on BIONET, write in Reader Service No. 122. See BIONET and IntelliGenetics in booth 33 at the Miami Winter Symposium.

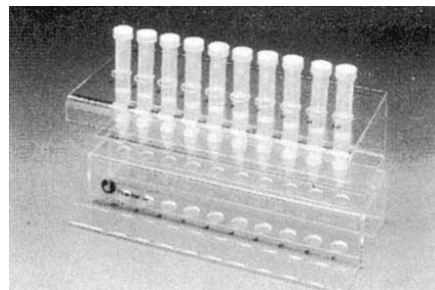
David Kristofferson is the BIONET Resource Manager, 700 East El Camino Real, Mountain View, California 94040, USA.

1. Zuker, M. & Stiegler, P. *Nucleic Acids Res.* 9, 133-148 (1981).
2. Mount, D.W. & Conrad, B. *Nucleic Acids Res.* 12, part 2, 811-824 (1984).
3. Lipman, D.J. & Pearson, W.R. *Science* 227, 1435-1441 (1985).

Straight from Miami

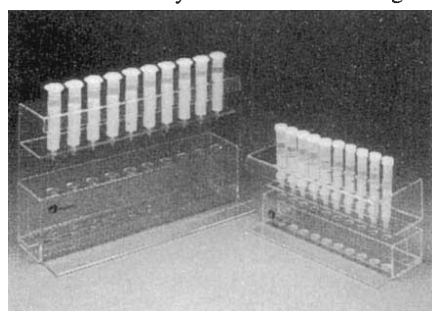
As participants converge upon Miami, Florida for the 19th Miami Winter Symposium, 9-13 February, Nature highlights products for studying peptides and oligonucleotides.

PHARMACIA has just introduced two new separation columns, and will be providing information on them in booths 1 and 2. The NICK columns are pre-packed with



For the speedy purification of nick-translated DNA.

Sephadex DNA Grade, and are specially designed for the separation of nick-translated DNA from unincorporated radiolabelled nucleotides, operating by gravity feed. Pharmacia reports that tests of NICK columns show that 90 per cent of applied radioactivity in a nick-translation mixture elutes in two well separated peaks. Maximum sample volume is 0.1 ml, with a total elution volume of 0.4 ml of purified DNA after 5 to 10 minutes. NICK columns are sold in 20 or 50 column packages for \$60 and \$130 (US), respectively. NAP columns by Pharmacia are designed



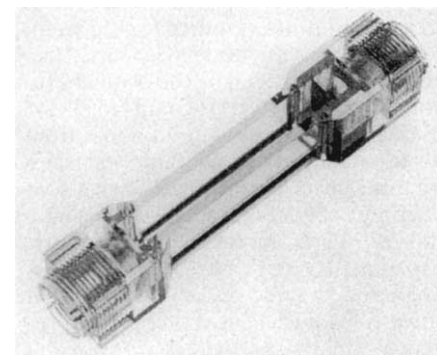
NAP columns purify DNA and proteins.

for the purification of DNA, proteins or oligonucleotides, and come in 0.5 ml, 1.0 ml and 2.5 ml sample volumes (Reader Service No. 100). Useful for desalting, removal of phenol or buffer exchange, NAP columns perform efficient purification in 15 minutes, according to Pharmacia. Also sold in 20 or 50 columns per package, NAP columns range in cost from \$74 to \$158 (US), for the 2.5 ml column size.

5 Prime→3 Prime, Inc. will be exhibiting its newest products in booth 4. Its \$110 (US) mRNA isolation kit is ready to use, consisting of an oligo dT cellulose column plus RNase free buffers, with enough reagents to swell, wash, load, elute and regenerate the column five times (Reader

Service No. 101). If RNase free buffers are all that's needed, the buffers alone are priced at \$45 (US). Disposable spun columns for radiolabelled and biotinylated probes are also sold by 5 Prime→3 Prime, costing between \$11.50 and \$60 (US) for 5 to 15 unit sizes, depending upon type.

Booth 5 will contain a display of Schleicher & Schuell's products, including its Elutrap chamber for the purification and concentration of macromolecules (Reader Service No. 102). The S & S Elutrap works by filtration in an electric field.



Separates the molecular wheat from the chaff.

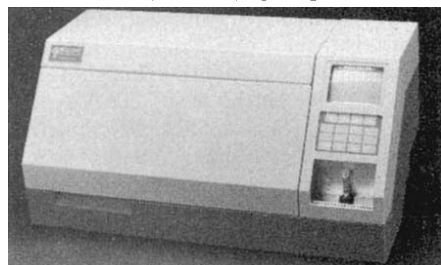
Sample molecules migrate into a trap between two membranes that are activated by electric current. The first membrane excludes large particles and other contaminants, and the second membrane retains the sample in the trap. Both membranes seal off the elution chamber in the absence of an electric current. According to Schleicher & Schuell, the Elutrap is ideal for the purification of proteins and DNA from gels, because it performs separations in the nanogram to microgram range, and has no effect on the sample's biological activity. The \$295 (US) Elutrap uses a standard power supply, and a horizontal electrophoresis chamber.

Custom oligonucleotide synthesis is the specialty of Midland Certified Reagent Company, in booth 21, who offers a cost-effective alternative for laboratories with only periodic requirements for synthetic DNA (Reader Service No. 103). Midland Certified Reagent Company uses phosphoramidite chemistry to prepare sequences, and guarantees to provide the correct oligonucleotide. Turn-around time is promised within ten working days, and is often as little as four or five working days. Midland Certified Reagent Company uses HPLC to purify the oligonucleotides up to about 30 nucleotides long prior to shipping, and provides the

elution profile as a permanent record of product purity. Prices for the service range from \$604 (US) for a 12-mer to \$1,360 (US) for a 60-mer, and are calculated per nucleotide.

Fisher Scientific's booths, numbers 19 and 20, will be filled to the brim with the Fisherbiotech line of **biochemicals, reagents and laboratory apparatus** (Reader Service No. 104). Items on display will include restriction endonucleases, DNA synthesis reagents, post-blotting apparatus, and hybridization membranes. For the cell biologist, Fisher Scientific will be exhibiting its prepared media, serum-free media, microcarriers, enzymes, and a disposable bioreactor.

Biosearch's new **DNA synthesizer**, Cyclone, will be whirling in booths 6 and 7 (Reader Service No. 105). Using the proven beta-cyanoethyl phosphoramidite



For foolproof DNA synthesis.

chemistry as well as the new H-phosphonate synthesis protocols, the \$18,900 (US) Cyclone employs preprogrammed memory cartridges, and can be adapted for new chemistries as they become available. Short fragments as well as sequences in excess of 100 bases can be prepared, with cycle times of 60 min for a 10 bp sequence. Conveniences of the Cyclone include a CRT display, prepackaged reagents for direct use without any necessary preparation, and a small benchtop footprint.

Du Pont, in booths 22 and 23, will have news of its new line of **peptide synthesizers** (Reader Service No. 106). The Coupler 2100 model has as its core Integrated Synthesis Logic, an artificial intelligence system that controls the preparation of 0.5 to 2.0 mmoles of peptide. The \$70,000 (US) Coupler 2100 provides continually updated systems status reports, as a built-in printer records the synthesis directions and parameters for each operational step. Using both BOC and Fmoc protocols, the Du Pont peptide synthesizer's reagents come in convenient pre-measured Coupler-Paks that can be loaded up to 49 at a time in any order in the carrier. At the appropriate time according to the programmed amino acid sequence, bar codes on the Coupler-Paks are read by a robotic arm, and the reagents are reconstituted with solvent and drawn into the instrument. For large-scale peptide synthesis,

the \$99,500 (US) Coupler 296 produces up to 500 g of peptide in a single run, according to Du Pont.

Electroelution of compounds separated on a gel improves the level of recovery. International Biotechnologies, in booth 24, has developed a Unidirectional Electroeluter that includes both protein and oligonucleotide purification (Reader Service No. 107). The \$475 (US) Model UEA works by using electrophoresis to drive the sample out of the gel matrix and into a high-salt cushion resting in a V-shaped channel. Once the DNA reaches the high-salt layer, it is trapped there in a small volume and can be directly ethanol-precipitated. International Biotechnologies reports oligonucleotide recoveries in excess of 90 per cent using the Model UEA, thus eliminating the need for dialysis tubing, phenol extraction or column purification in the DNA fragment preparation process.

Boehringer Mannheim Biochemicals, in booths 25 and 26, has a plethora of **reagents, enzymes, and kits** for the molecular biologist (Reader Service No. 108). The list includes two new restriction endonucleases, *FokI* and *SpeI*, a set of restriction enzyme buffers, and a T_4 -DNA polymerase with 5'→3' polymerase, and 3'→5' exonuclease activity. For purifying labelled DNA and RNA, Boehringer Mannheim sells prepacked, preswollen, prespun columns that it claims achieve 90 per cent recovery of labelled DNA, and 80 per cent recovery of labelled RNA, each in a 6 min, 2-step procedure. A kit for labelling DNA using random oligonucleotide primers is also available, that performs 50 labelling reactions containing 0.01-2 µg DNA, using either radioactive or chemically modified nucleotides.

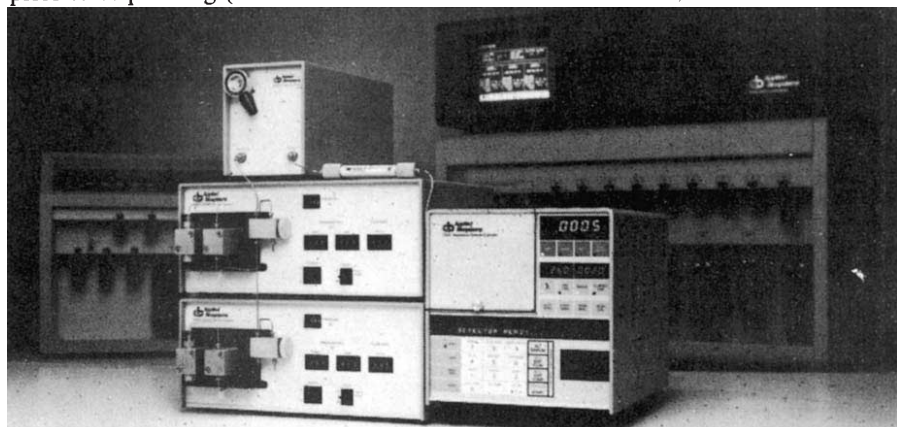
Booths 31 and 32 will be filled with Applied Biosystems' range of instruments for **automated molecular biology**. Among the choices presented will be four separation systems for the isolation and purification of proteins, peptides and polynucleotides prior to sequencing (Reader Service No.

Forensic biotechnology

BioTechnica Ltd, the Cardiff-based biotechnology company, has thought up a unique way to harness the information storage capability of DNA. It has developed a labelling system, using DNA fragments, to protect companies against illicit marketing and counterfeiting of their products (Reader Service No. 110). BioTechnica's BIO-TAG system consists of randomly synthesized sequences of DNA, whose code is known only to the manufacturer, affixed to a tag. When the authenticity of the item needs to be confirmed, the DNA fragment is sequenced. BIO-TAG can be applied to a variety of materials and has the ability to accommodate varying security levels, and the coded DNA tag can be made invisible, if necessary. In addition to protecting against counterfeits, BioTechnica sees a market for its product in tracking the flow of documents to be held within a secure system, and in tracing identity documents or other valuable items such as passports, bank bearer bonds, security certificates or works of art.

109). The \$36,000 (US) Model 130A protein purification system performs sample preparation and analysis for low picomole levels of proteins and peptides, using microbore (1 mm i.d.) cartridge columns to achieve elution volumes of less than 50 µl. For cases where the amount of protein or peptide under study is not limited, the \$19,000 (US) Model 150A protein separation system uses standard bore pumping to provide speed and resolution equivalent to the Model 130A, at a much lower cost. Post-synthesis purification problems are cleaned up using the \$21,700 (US) Model 151A for synthetic peptides, and the \$20,800 (US) Model 152A for synthetic polynucleotides.

Haake Buchler Instruments also has a new **DNA sequencer**, that can be seen along with its other Ephortec wares for molecular biology in booth number 28 (Reader Service No. 111). Haake Buchler's gel electrophoretic DNA sequencer comes in two sizes, 100 cm and 45 cm.



Applied Biosystems has machines to automate protein and polynucleotide purification processes.

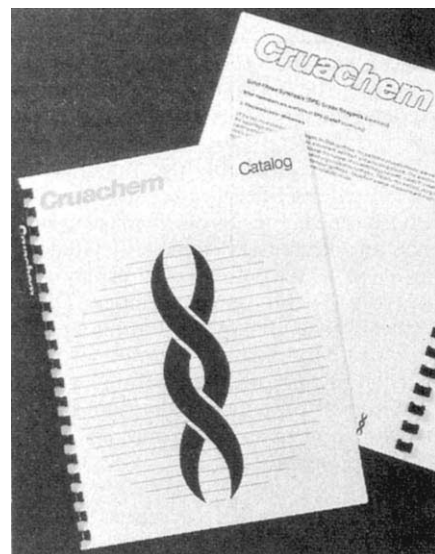
Both systems employ an aluminium platen that Haake says assures even heat distribution, eliminating the "smile" effect. The sequencers have a thermoregulated design, and can be set for high-speed, high-voltage runs or at lower voltages for overnight runs. The separating gel for the \$1,885 (US) 100 cm DNA sequencer is 95 cm in length, allowing over 200 nucleotides to be read at a single loading, and is compatible in size with the 22 × 100 cm HBI Transfilm for autoradiography. The \$1,595 (US) 45 cm sequencer's gels can be used with standard 35 × 43 cm X ray films.

In addition to its Retro-Tek AIDS virus kits and probes, Cellular Products will be showing its Cellkines line of **human immune cell culture products** in booth 30 (Reader Service No. 112). Cellular Products has a full line of growth factors, including human T-cell growth factor, human B-cell growth factor and human platelet-derived growth factor, which sell for \$47.50 to \$195 (US), depending upon

size desired. For detecting the HTLV-1 core protein, p19, Cellular Products offers anti-p19 monoclonal costing \$175 (US) for 100 tests. Human immune (gamma) interferon is also available from Cellular Products, for purely *in vitro* diagnostic use.

Seikagaku America, in booth 39, will be showing its **molecular cloning products** (Reader Service No. 113). At the top of the list will be Seikagaku's reverse transcriptase, for the synthesis of complementary DNA to mRNA. Seikagaku sells the reverse transcriptase in 2,000 unit sizes for \$240 (US), and in 5,000 unit sizes for \$500 (US), and defines a unit as the amount of enzyme which incorporates one nanomole of dTMP into acid-insoluble product in 10 minutes at 35°C.

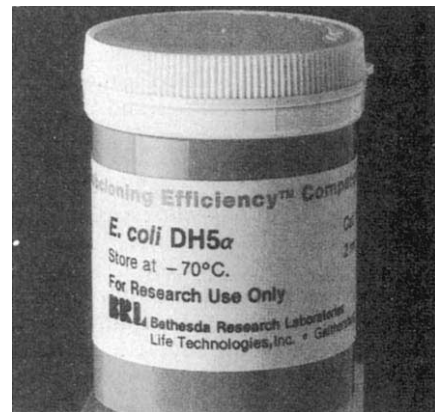
The PS 200 automated DNA synthesizer will be dominating attention in the Cruachem booth, number 40, but the company representatives are also likely to be showing off their **new 1987 catalogue**



The book on DNA synthesis reagents.

(Reader Service No. 114). The catalogue features solid phase synthesis (SPS) grade chemicals and reagents, including Cruachem's CE cyanoethylphosphoramidites for "easy on the nose" DNA synthesis. Cruachem's One Shot phosphotriester starter kit simplifies manual or semi-automated DNA synthesis by using single-use packs containing enough material for 100 coupling reactions on a 1 micromolar scale. ColorTag monomers provide visual verification of sequence during synthesis, so unsatisfactory syntheses can be stopped before costly reagents are wasted.

Made just for researchers performing routine subcloning, GIBCO/BRL in booths 34 and 35 offers Subcloning Efficiency **competent cells** (Reader Service



Increases the level of uptake of plasmid DNA. No. 115). GIBCO/BRL says use of its competent cells yields consistent numbers of greater than 1×10^6 transformants per microgram of monomer plasmid DNA, at less than one-eighth the price per reaction of other commercially available competent cells. The DH5 alpha *recA1* marker ensures the stability of inserts, and its *endA1* marker improves the quality of plasmid DNA prepared from mini-preps. GIBCO/BRL's competent cells cost \$37 (US) for a 2ml sample.

Molecular biology becoming more fashionable?

Dr Ruth Kavenoff, a cell biologist studying chromosome structure at the University of California, San Diego, is bent on bringing the beauty of science to the general populace. Through her work with electron microscopy, she has imaged "sprung" *E. coli* DNA molecules, confirming the DNA supercoiling model set down by Worcel and Burgi¹. Her technique employs an aqueous salt solution, instead of the usual formamide solution, and the result is a much clearer view of the DNA loops involved in the supercoil. The aesthetic appeal of the resulting spread molecule led her first to produce wall posters of the image to decorate the laboratories of friends and associates. From there, she went on to print her images on more popularized items, such as postcards and T-shirts, and her company, Designer

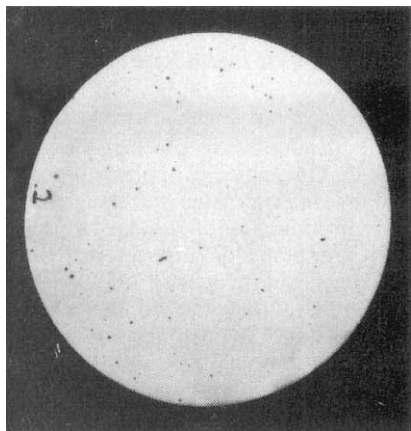
Genes, was born. Her range of products, that now includes labcoats, sweatshirts, and totebags, all come with an explanation of the image they bear, and a bit of background on DNA in general (Reader Service No. 116). Other images are currently in the pipeline, including micrographs of chloroplast DNA (GreenGenes), antibody mRNA and DNA (A Splice of Life), and flu virus DNA (The Usual Suspect). In this manner, Dr Kavenoff hopes to increase the level of knowledge of molecular biology held by the hoi polloi. It is her opinion that "people are reluctant to fund research they don't understand," and she seeks to improve this understanding. A variety of DesignerGenes products are now distributed by Carolina Biological Supply Company, Burlington, North Carolina, 27215.

1. Worcel, A. & Burgi, E. J. *molec. Biol.* 71, 127-147 (1972).



Dr Kavenoff seated by the source of her images, wearing one of her DesignerGenes shirts.

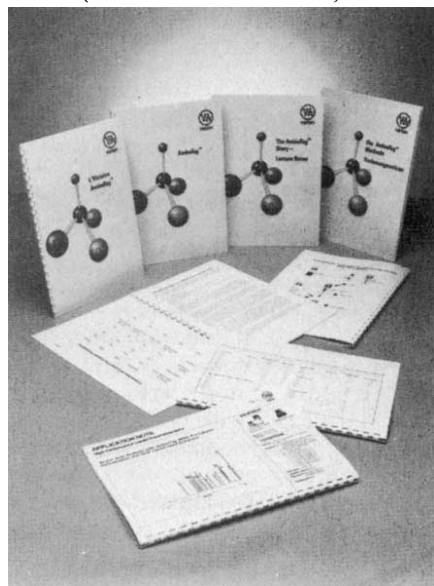
Bio-Rad's Express-Blot alkaline phosphatase kit is a sensitive, **non-isotopic method for detecting specific proteins** expressed in a few plaques out of a large library (Reader Service No. 117). Based



Highlights poorly expressed proteins.

on a goat anti-rabbit alkaline phosphatase conjugate, Bio-Rad says the Express-Blot kit is useful for detecting poorly expressed proteins, or proteins to which only low titre or low affinity antibody is available. The \$195 (US) kit comes complete with all components and protocols needed to conduct the complete plaque lift and detection procedure. Express Blot also works in colony lift applications and with other expression systems in which rabbit anti-serum specific for the desired protein is available.

Lecture notes on amino acid analysis using Amino Tag are now available from Varian (Reader Service No. 118). Written



Varian's AminoTag lecture notes tell the full story of amino acid analysis.

by C. Bruton of Trinity College, Cambridge, United Kingdom, the notes offer an overview of the importance and history of amino acid analysis, and outline the performance of various pre- and post-column derivatization reagents.

Protein structure can be studied with a new software package developed by the University of Michigan in Ann Arbor, Michigan, United States (Reader Service No. 119). The \$400 (US) MSEQ program (\$200 for non-profits) is written for the IBM microcomputer family, and features various approaches to the quantification and understanding of amino acid sequence, secondary structure, transverse hydrophobic moments and hydrophobicity. MSEQ produces "cartoons" integrating secondary structure predictions with hydrophobicity calculations based on amino acid sequence, that can be printed out on a digital X-Y plotter. The program is provided with an archive diskette containing 111 sequences from most of the major well-characterized gene families, plus detailed instructions for use.

Synthetic Genetics specializes in **custom DNA and peptide synthesis services** (Reader Service No. 120). For \$10 (US) per



For occasional peptides and oligonucleotides. nucleotide, Synthetic Genetics will produce up to 100-mer unpurified oligonucleotides, in phosphorus-modified and fluorescent labelled form, if desired. Synthetic Genetics also generates guaranteed-sequence polypeptides, at \$95 (US) per amino acid, and backs its peptide with analysis by TLC, UV-absorption spectroscopy, HPLC and amino acid analysis. Other services, such as conjugate formation and HF cleavage for peptides, and deoxyinosine substitutions and enzyme labelling for oligonucleotides, are also offered.

Clontech Laboratories, Inc. has begun a **custom gene expression service** (Reader Service No. 121). Using *Escherichia coli*, *Bacillus subtilis*, yeast, mammalian or retrovirus expression vectors. Clontech will clone and express both known and unknown DNA sequences. The service is available for approximately \$2,800 (US) for expression of a known sequence.

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal and apply only within the country indicated.

ADVERTISEMENTS

PEPTIDE & PROTEIN RESEARCH

**HIGH QUALITY
CUSTOM
PEPTIDE SYNTHESIS**

- GUARANTEED PURITY
- FULL ANALYSIS PROVIDED
- SULPHATED PEPTIDES
- HYDROPHOBICITY PLOTS
- FAST DELIVERY
- KEEPEST PRICES

For further details or a specific quotation Contact Dr M.S. Morris

PEPTIDE & PROTEIN RESEARCH CONSULTANTS
1 Sheepcote Road
Eton Wick, Windsor
Berks. SL4 6JA
United Kingdom (0753) 859792

Reader Service No.21

SINGER INSTRUMENTS
MICRO-TOOL MAKING?
then you need the
SINGER MICROFORGE

Write to: Singer Instrument Co Ltd, Treborough Lodge, Roadwater, Watchet, Somerset TA23 0QL, England. Tel: (0984) 40226. Telex. 337492 Comcab G.

Reader Service No.50

LIPOSOMES NEW
REPRODUCIBLE — HOMOGENEOUS —
EXTREMELY RAPID

Our new LIPOSOMAT guarantees rapid (5ml within 60 minutes) preparation of uniformly sized unilamellar liposomes. Liposome size is selectable between 25 and ca. 600 nm diameter. Sample volume is normally 5-10ml or up to 200ml if used in combination with an additional instrument. Lipid concentration can be up to 300 mg/ml.

BIANORM
POB 126, D-8000 Munich 65, FRG

Reader Service No.47

EMPLOYMENT

The threat and promise of robots

Richard Pearson £140

Is the introduction of robots into industry increasing efficiency and so creating jobs, or is the human worker soon to become a thing of the past?

ALTHOUGH robots in one form or another have been around for many years, it is only in the second half of the 1980s that their widespread use has come into prospect. In terms of the application of basic electronics in products and processes, West Germany is the leader in Europe with Britain second, ahead of France. Although the rate of take up has been increasing over the past five years there is a long way to go — in 1985 only half of Britain's factories were using microelectronics.

But when it comes to robots, Britain seems to be lagging rather further behind its international competitors. Not only do Japan and the United States use more robots, but West Germany with an industrial base of similar size, has two and a half times as many robots in use. The rate of increase of use in Germany is also far greater and in 1985 the increase in the one year was as great as the total number installed in the whole of Britain in the previous years combined. France and Italy are also ahead in their use of robots, whereas Swe-

den in part to their greater wish to learn about the technology, as well as their greater financial resources and in-house expertise. Perhaps the most worrying finding is that robots are three times as common in overseas-owned companies as in British-owned ones. Although the final decisions to buy robots were normally taken by "head offices" or "company boards" the original ideas and motivation were more likely to come from plant management.

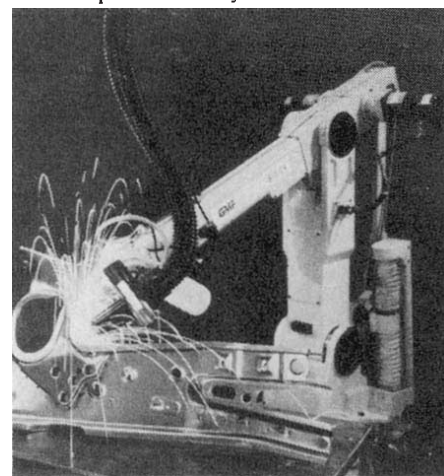
In 1982 the government established a Robotics Support Programme which initially included grants for feasibility studies, towards the purchase of robots, and for research and development work by robot manufacturers. The scheme has been modified and renamed several times since, but grants are still available. Although the majority of users undertook feasibility studies before introducing robots, only one in four received a grant. One in six were not aware of the availability of the grants, for the rest the amount of money available was too small to be worth the trouble of applying. Rather more firms got grants towards the costs of the robots, half of those who were eligible, and for many the availability of these grants was an important, and often decisive factor in their investment decision.

The likelihood of opposition to new technology amongst employees — especially in its visible form as a robot — is often thought to be a barrier to its introduction. But this proved an unfounded fear amongst companies surveyed by the PSI, and shopfloor opposition was reported in only 2 per cent of the factories, with a similar percentage reporting opposition from top management. Before the robots were introduced 42 per cent of users reported their workforce to have favourable attitudes towards them, rising to 71 per cent after their introduction. Unfavourable attitudes, short of opposition, were reported in 9 per cent of cases before their introduction, falling to 4 per cent after. Four out of five users consulted their staff before introducing robots and this was closely related to the most positive of the attitudes reported. Half of the users said staff benefited from improved working conditions and safety.

A major concern for many is the impact on employment levels. This is not always easy to determine directly as the introduction is usually accompanied by other changes in products and factory organization, many of which may have been possi-

ble independently. In practice the study found that the majority of plants reported no change in employment levels as a result of the introduction of robots. However, one in four plants did report a reduction, this being especially true of the larger plants and those using robots over a longer period of time. The average loss in these plants was about 8 jobs each although there were compensating job gains in one in twelve plants. The PSI authors estimate there was a net overall loss of 700 jobs in all the plants using robots in Britain although direct redundancies were rare.

Looking to the future, 60 per cent of users expected to buy more robots in the



Arc welding by Metatorch, a 'second generation' robot manufactured by Meta Machines of Abingdon. (Science Photo Library.)

next two years whereas it was in only a few plants that they said their existing robots had not been worthwhile. Overall, it is estimated that the robot population could grow from 3,200 in 1986 to between 4,200 and 7,200 in 1988, the lower figure reflecting the recent slowdown in growth and the higher figure being based on the optimistic outlook reported by the users themselves. The latter figure compares with recent growth in West Germany.

Clearly job losses in manufacturing are going to continue regardless; investing in new technologies is not going to be the major cause but its introduction can, however, improve competitiveness and help to stem the tide and must continue to be a key priority for companies, their workforces and government alike. □

Richard Pearson is at the Institute of Manpower Studies, Mantell Building, University of Sussex, Brighton BN1 9RF, UK.

The advance of robots in British industry

Year	Robot users	Robots
1981	100	371
1982	150	713
1983	220	1,152
1984	350	1,753
1985	560	2,623
1986	740	3,208

Source: *Robots in British Industry*, Policy Studies Institute, 1986.

den with a far smaller industrial base has only slightly fewer robots than Britain.

Despite the recognized problems of definition, it has been estimated in a report from the Policy Studies Institute (PSI) that at the beginning of 1986 there were nearly 750 factories in Britain with robots, and between them they had about 3,200 robots, averaging about 4 per factory. While the total numbers have increased more than sevenfold since 1981 this has been from a low base — and the rate of increase in 1986 was lower than in the previous year (see table).

As might be expected, the robot users are to be found predominantly in the more sophisticated industries, particularly vehicles, aerospace and electronic engineering, with rather fewer in mechanical engineering. The robots are also much more common in large plants and in those using other forms of new technology. The larger companies' greater use of robots is put